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CLINICAL STUDIES WITH THE BALLISTOCARDIOGRAPH; IN CONGESTIVE FAILURE, ON DIGITALIS ACTION, ON CHANGES IN BALLISTIC FORM, AND IN CERTAIN ACUTE EXPERIMENTS.*

By Isaac Starr, M.D.,

HARTZELL RESEARCH PROFESSOR OF THERAPEUTICS, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PA.

Work on the perfection of the ballistocardiograph[11] and the interpretation of its records has taken two directions. One of these might be termed the physiologic, the other the empirical approach. In the first it is aimed to discover what physiologic functions can be measured and how accurate the measurement is. This part of the work has consisted of a mathematical attack on the problems involved,[7,11] animal experiments,[11] and comparisons of results obtained by the ballistocardiograph with those secured by other cardiac output methods;[2,11] and work of this type is far from final completion. But, even if exact measurements could be made, it does not follow that the relationship between the physiologic aspects of the circulation and the clinical aspects of the cases would always be clear. Therefore, the empirical approach is equally important and this paper is a report of our progress in that direction. The data were drawn from over 1000 records made on many hundreds of patients during the last 5 years.

The purpose of this presentation is to acquaint readers with the characteristic appearance of ballistocardiograms in certain familiar clinical conditions, and with enough of the physiologic background to aid in drawing conclusions from such records. I propose to set forth the clinical possibilities of the method by showing how the results illuminate two questions much debated in recent years, the state of the circulation in congestive failure, and the

* A considerable part of the data contained in this paper was given, as a preliminary report, before the Association of American Physicians in May, 1941.

action of digitalis; and by demonstrating several approaches to new clinical problems which the ballistocardiograph makes possible.

The ballistocardiograph is simply a suspended table, braced to prevent motion in any direction but the longitudinal. Motion in that direction, opposed by a strong spring, is magnified by an optical system and photographed. A normal young adult lying on the table gives a record like that shown in Figure 1, Row 1, the main features of which can be easily understood. The contracting heart expels its blood headward initially and, for the same reason that when you fire a gun it kicks you in the shoulder, when the heart's blood goes headward the body is propelled feetward. An instant later this blood strikes the arch of the aorta and the curve of the pulmonary artery and its headward movement is in large part arrested and, simultaneously, blood in the thoracic and abdominal aortæ starts to move feetward. The forces now generated drive the body headward and one can easily see that the larger the amounts of blood expelled the greater would be the impacts. Large subjects give greater impacts than small ones and, if any subject submits himself to conditions known to stimulate cardiac output, such as exercise or adrenalin, a marked increase in the size of the recorded impacts follows invariably.

The view that the amplitude of the record is a function of the cardiac output is supported by these simple facts as well as by comparison with other cardiac output methods. Indeed, this is a field which, if foreign to clinicians and strange to physiologists, is an old stamping ground for physicists and engineers, the theory being directly derived from Newton's laws of motion. However, enough is known of the difficulties to make me believe that this

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LEGEND FOR FIG. 1.

FIG. 1.—Ballistocardiograms reduced to one-half actual size and taken after a 15-minute rest period, never within 2 hours of a meal, with the subject horizontal. In the time record the largest intervals are 1 second.

Top. Record of a normal medical student. Age 23; height 5 ft. 10 in.; weight 155 pounds.

Rows 2 and 3. Records of S.S. Age 50, height 5 ft. 9 in., weight 125 pounds. Rheumatic heart disease, mitral stenosis and regurgitation, tricuspid regurgitation (?), auricular fibrillation. Row 2 while in failure; the large downward deflection at each end is diaphragmatic. Row 3 after recovery from it. This patient has been receiving digitalis for a long while and it was continued in the hospital.

Rows 4, 5 and 6. Records of C.J. Arteriosclerotic heart disease. Row 4, in failure. During Cheyne-Stokes breathing; time of ejection marked by dots under the record. Cardiac output per minute deviates from the expected normal by at least +22%. (Normal limits ±22%.) Row 5, still in failure, during labored normal breathing; position of diaphragm noted on record; the large impacts are all diaphragmatic. Position of ejection indicated. Cardiac output −9%. Row 6, out of failure. Cardiac output +22%.

Row 7. M.C. Hypertensive heart disease. *Left*, while in failure; note the unusually prominent respiratory variations from the labored breathing. Cardiac output in middle of normal range. *Right*, patient out of failure with striking clinical improvement. Cardiac output −39%.

method, like most clinical methods, is of low accuracy and one should regard it in this way, contrasting its results not only with the ideal, but also with the ordinary clinical procedures which leave

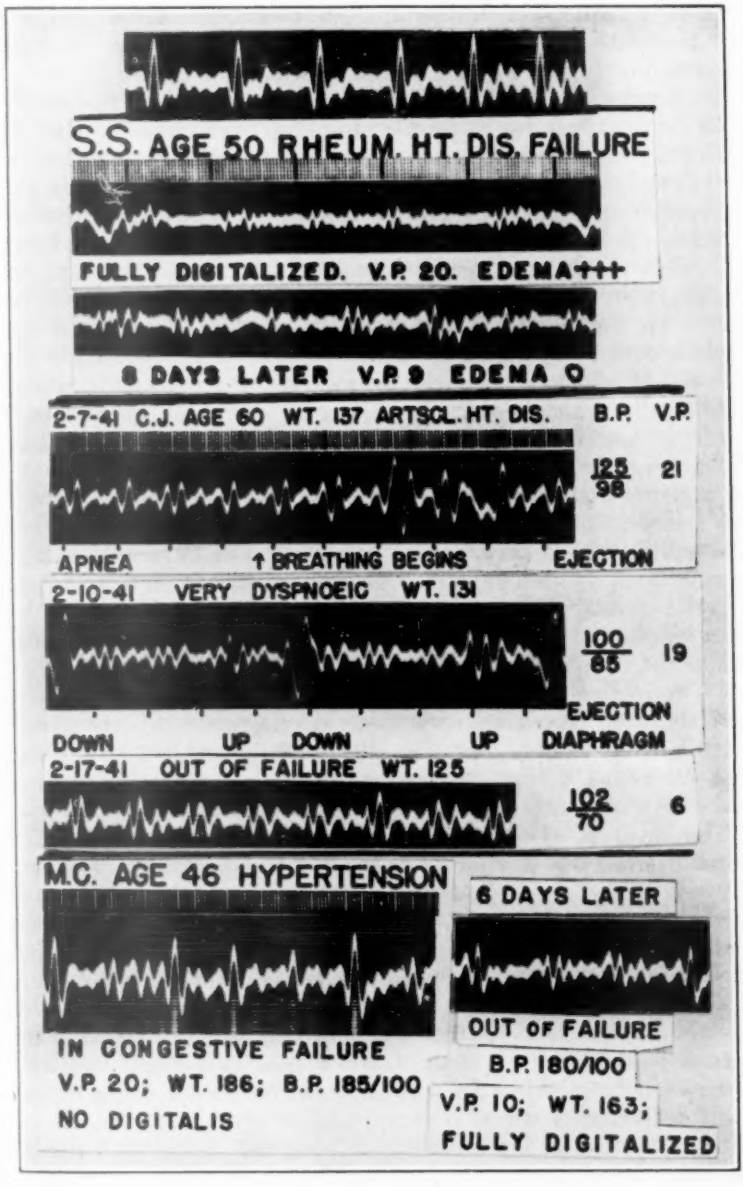

FIG. 1.

one with practically no idea at all concerning the amount of the circulation.

The method is well suited to use in the clinic. Nothing is required of the patient save that he lie relaxed on the table. No especial training is required of the operator and records can be secured in a few minutes. Indeed, the extremes of abnormality can often be identified by a glance at the flickering light spot.

Taking Records. All records were secured on subjects who had been lying at rest for at least 15 minutes. No record was taken within 2 hours after a meal.

On the Inspection of Ballistocardiograms. A chief hazard in the interpretation of the records by inspection alone, as readers would do, rather than by measurement and calculation, lies in the tendency to neglect the size and age of the subject. Large persons have far larger absolute cardiac outputs than small ones and the amplitude of the ballistic record is related to the absolute value, while the decision concerning normality depends on the output referred to the size of the subject. Also elderly subjects, even though healthy, give smaller impacts than their juniors. Also the heart rate is an important factor both directly, and indirectly by indicating a change in the duration of systole. Therefore to judge normality by a glance at the record requires considerable experience, even if age and weight are known, and direct comparison of the records of two subjects is handicapped by the same difficulties. However, records made by a single subject, at times reasonably close together, may be directly compared with confidence and in this paper the chief emphasis is on just such comparisons. So inspection should suffice the reader to justify, or reject, the main conclusions.

On examining the records, one must divest himself of a tendency, derived from experience with electrocardiograms, to concentrate attention on details. Any movement of the patient affects the ballistic record, so that artefacts are common. Only the features which are constantly repeated deserve any attention.

The *shape* of the record should engage attention first. The normal form (Fig. 1, Row 1) shows a sharp downward deflection (I wave) immediately followed by a sharp upward deflection (J wave) at each systole. These waves stand out in contrast to the smaller and more variable waves in diastole. There is evidence that the shape of the systolic record is a reflection of the changes in blood velocity in the aorta. The normal heart expels blood with a snap, *i. e.*, maximal velocity is attained early in systole, so in the normal ballistocardiogram the I and J peaks are sharp and they occur early in systole. In some diseased hearts the initial downward deflection (I wave) is inconspicuous and the J peak moves back in systole, changes consistent with the view that maximal expulsion velocity is not attained until late in systole in such cases.

The *size* of the record should be assessed next for, with reserva-

tions to be discussed, the amplitudes of the first downward and largest upward deflections, I and J waves, depend on the magnitude of the cardiac output. The respiratory variation is always present, unless the breath is held, the size of the complexes increasing during inspiration, diminishing during expiration. Cardiac output is estimated from measurements of representative high and low complexes.[11]

In special circumstances other factors enter into the estimation of cardiac output from the size of the ballistic record. If the form is abnormal the evidence indicates that the cardiac output was larger than the amplitude of the ballistocardiogram suggests. When the rhythm is abnormal, uncertainty exists concerning the best value to use for the duration of systole, although I believe that this error will be small if data from several complexes are averaged.

In other cases the difficulties are inherent in the subject rather than the method. If the heart valves leak, the ballistocardiogram records the sum of the blood regurgitated and that contributing to the circulation, and this must be kept in mind in interpreting the records. In dyspneic patients the movements of the diaphragm create impacts which, if frequent and violent enough, may mask the cardiac complexes.

Indeed, all the difficulties are likely to be at their maximum in the investigation of advanced cardiac disease, which is a major subject in this presentation. Because of these various difficulties cardiac output cannot be estimated with confidence in several of the cases to be discussed. Nevertheless, the direction of the changes taking place in their circulation is obvious at a glance, and it is to these changes that I wish to direct attention.

Data on the Circulation in Congestive Heart Failure. This problem has always presented special difficulties. Methods of estimating cardiac output based on the use of gases are hazardous due to changing abnormalities in the lungs. The ballistic method avoids this difficulty but is subject to the others discussed before and they must be kept in mind when records are interpreted.

Figure 1 shows the record of S.S., a case of chronic rheumatic heart disease and mitral stenosis and regurgitation, and possibly tricuspid regurgitation as well. There was auricular fibrillation also and the records in Figure 1, characteristic of this condition, show the impacts irregularly spaced and varying somewhat in form from beat to beat.

In the first record the cardiac impacts are minute, the larger downward deflections at each end of the record are diaphragmatic impacts from the labored breathing; after improvement the latter are seen no more.

Recalling that, because of the regurgitation of blood through the leaking valves, the impacts would indicate a larger circulation than was actually present, one is left with no doubt that the circulation

during failure was far subnormal. After recovery from failure the impacts were larger. It is scarcely conceivable, especially in the absence of an active lesion or a change in physical signs, that a greater proportion of the blood was being regurgitated at this time, so we conclude that clinical improvement coincided with a return of the circulation towards normal.

A similar result was seen in patient W.F. (Fig. 2) who was in normal rhythm and without evidence of leaking valves, so his cardiac output can be estimated with more confidence. In his first record, obtained during congestion, the cardiac complexes can hardly be identified, the two large downward deflections being diaphragmatic. The contrast with later records, especially that obtained one year later, is striking. The circulation after recovery of his health was far larger than when he was seriously ill. I could cite many similar cases.

But exceptions are common. C.J. (Fig. 1) was also in normal rhythm and without evidence of leaking valves. This patient was in Cheyne-Stokes' respiration when first tested and the record shows the beginning of breathing after an apnea of about 20 seconds. The first breath was a small one but the coincident improvement in the circulation is very striking. During the larger and more forceful breaths which followed, the diaphragmatic impacts so distorted the record that estimation of cardiac output at that time was hazardous. Nevertheless, even during the apnea the circulation was not subnormal. In this instance congestive failure was not associated with subnormal circulation.

When clinical improvement began, the Cheyne-Stokes' breathing disappeared. The next record shows conspicuous diaphragmatic

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LEGEND FOR FIG. 2.

FIG. 2.—Ballistocardiograms reduced to one-half actual size.

Rows 1 to 6. W.F., age 49, height 5 ft. 4 in. Admitted in congestive failure; the etiologic diagnosis was never clearly established. There was no evidence of valvular disease. Lues suspected, was never proved. The digitalis total dose is given in grains. Venous pressure is in cm. H_2O. Row 1, during failure, the large downward impacts are diaphragmatic; the cardiac complexes so small they can hardly be identified with certainty. He received a small dose of digitalis just before the record was made. Row 2, after rapid recovery from failure; note great increase in cardiac impacts and small dosage of digitalis. Row 3, the stimulation does not persist but clinical improvement continues. Row 4, an abnormality of form, the double I type, is present. Row 5, the record continues to improve, the larger complexes of the respiratory cycle are now normal, the smaller still abnormal. Patient was discharged the next day. Row 6, surprising improvement in record in a year's time. Patient is working steadily and considers himself cured.

Rows 7 and 8. M.B. Male. Age 20, height 5 ft. 5 in., weight 139 pounds. Traumatic arteriovenous communication right thigh, otherwise healthy. Row 7, before occlusion. Blood pressure 126/66. Cardiac output at normal average. Row 8, during occlusion of the circulation to the abnormal leg. Cardiac output −17%. Other control records obtained after release of the occlusion and during occlusion of the normal leg were very similar to that of Row 7 and have not been reproduced.

impacts from the regular labored breathing. Between them the cardiac impacts can be seen, smaller than in the previous record, but the cardiac output was still within normal limits. With com-

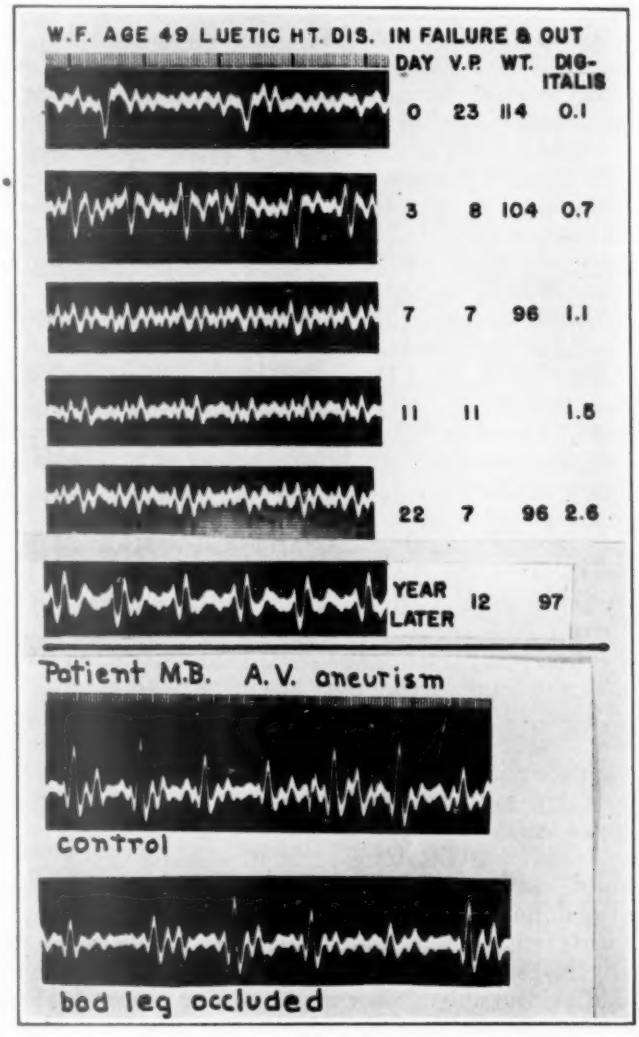
W.F. AGE 49 LUETIC HT. DIS. IN FAILURE & OUT
DAY V.P. WT. DIG-ITALIS
0 23 114 0.1
3 8 104 0.7
7 7 96 1.1
11 11 1.5
22 7 96 2.6
YEAR LATER 12 97
Patient M.B. A.V. aneurism
control
bad leg occluded

FIG. 2.

plete recovery from failure the circulation increased. If we had only the last two records we might conclude that clinical recovery was caused by the increase in circulation, but the evidence of the first record forbids this deduction.

In the case of M.C. (Fig. 1), the evidence is clear that his cardiac output during congestive failure was much larger than that present after he had recovered from it.

Equally impressive is the fact that many patients with profoundly subnormal circulations due to many causes do not have congestive failure. Cases I.P. (Fig. 3) and S.L. (Fig. 5) are examples.

In summary, our evidence concerning the circulation in congestive failure coincides with the great bulk of data in the literature. The majority of such cases have subnormal circulations, and improvement of the circulation often coincides with clinical improvement, as repeatedly found by Stewart.[13] On the other hand, this relationship is not invariable, as emphasized by Harrison,[4] and any idea that the symptoms are the direct mechanical consequence of a diminished circulation must be abandoned.

Data on Digitalis Action. Patient I.P. (Fig. 3) had arteriosclerotic heart disease with auricular fibrillation without any evidence of valvular disease. She had never been in failure and had received no digitalis for over a month. On admission the cardiac impacts were so small that it is hard to be certain where systole was. A week in bed without digitalis resulted in some slight clinical improvement, and the systolic impacts can be seen a little more clearly at this time. Digitalis given for 3 days coincided with further clinical improvement and the record shows a very marked increase in the size of the impacts at that time. It should be noted that the amount required to produce this effect was less than that calculated to produce the drug's full effect; the clinicians in charge did not regard the patient as "digitalized" when the third ballistocardiogram was taken. The patient was certainly digitalized 2 days later when the fourth record was taken. At this time the pulse rate had diminished and the circulation was certainly smaller than it had been 2 days before, although probably larger than on admission. The conspicuous effect is the transient stimulation of the circulation early in the drug's action.

A similar transient stimulation, demonstrated after 0.7 gm. of digitalis in patient W.F., is seen in his second record (Fig. 2), although the deep downward "K" waves give the impression that the cardiac output is larger than is actually the case.

This early stimulant action of digitalis can be demonstrated frequently in both normal rhythm and auricular fibrillation, but by no means invariably. Since it is a passing phase of the drug's action, it is to be expected that the records would not always be secured at the right time. Transient stimulation of the circulation after digitalis has appeared occasionally in tests with the older cardiac output methods in the hands of Stewart,[12] Harrison,[4] and our own group;[10] but, in the belief that digitalis had no effect until larger doses had been administered, most of the experiments were not timed to demonstrate it and it has never received the emphasis it deserves.

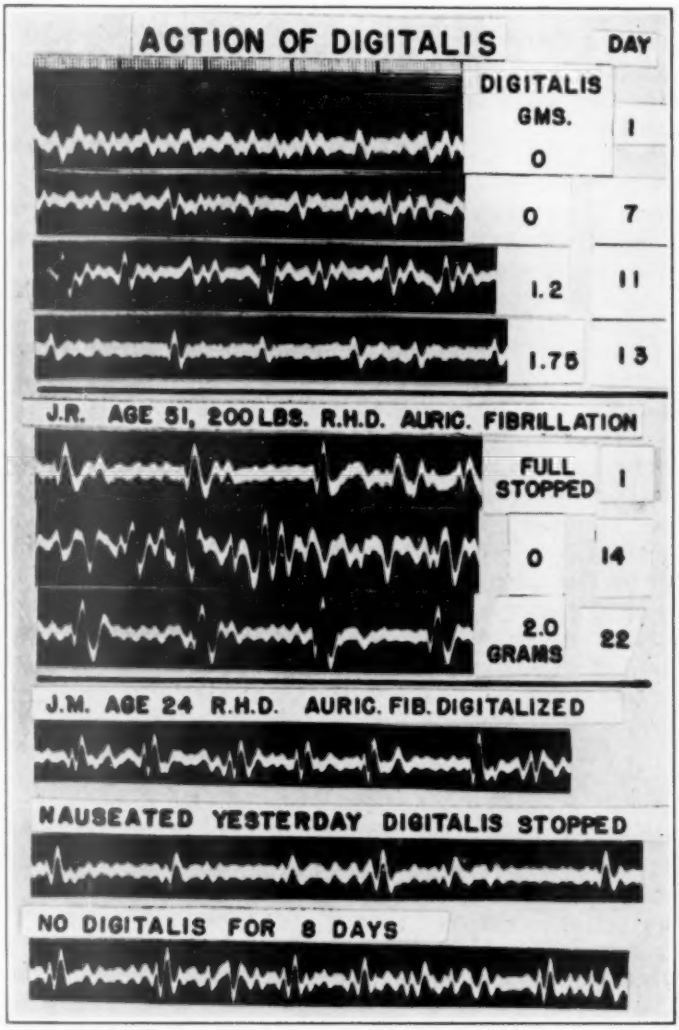

FIG. 3.—Ballistocardiograms reduced to one-half actual size. The top time record, largest interval 1 second, applies to all records.

Rows 1 to 4. Records of I.P., age 65, height 5 ft. 2 in., weight 128 pounds. Arteriosclerotic heart disease. Auricular fibrillation, Class II A. On admission the patient had had no digitalis for over a month. There were no signs of failure, but the patient was weak. Some weeks later operation disclosed a hypernephroma. The digitalis column gives the total dose administered.

Rows 5 to 7. J.R., age 51, height 5 ft. 10 in., weight 200 pounds. Rheumatic heart disease, mitral stenosis, auricular fibrillation, Class II A. No signs of failure. Fully digitalized on admission, the drug was omitted for 14 days, then given once more. There was no obvious change in his clinical condition during this test. Note the distorted record when not under the influence of the drug.

Rows 8 to 10. Records of J.M., age 24, height 5 ft. 9 in., weight 130 pounds. Rheumatic heart disease, mitral stenosis, auricular fibrillation. Row 8, Jan. 4, just recovered from congestive failure, fully digitalized and on maintenance dose. Row 9, Feb. 6, giving signs of too much digitalis. Row 10, Feb. 12, after 8 days without the drug.

When too much digitalis is given, the ballistocardiograph demonstrates that the well-known slowing of the rate is accompanied by a diminished size of the individual complexes (J.M., Fig. 3), a combination which must result in a profound depression of the circulation.

Finally, full or excessive dosage of digitalis has a characteristic effect on the ballistocardiograms which one can often identify at a glance. If the patient has auricular fibrillation the drug causes conspicuous flattening of the diastolic part of the ballistic record between the prominent systolic complexes. The records of patient J.R. (Fig. 3) digitalized, taken off the drug and then digitalized again, show this phenomenon well and it can be seen in the records of I.P. and J.M. (Fig. 3) also.

We find, therefore, that the action of digitalis consists of a stimulation early in its action at a time before the most obvious clinical effects can be recognized. This phase is temporary and it is followed by a period when slowing is prominent if there is auricular fibrillation. Overdosage is characterized by a profound depression of the circulation. Perhaps readers will find the initial stimulation surprising, for it has often been taught that the drug has no detectable effect before the full picture of digitalization sets in. But pharmacologists would be less surprised, for the stimulation demonstrated by our records is analogous to Cushny's first stage of digitalis action,[3] the transient stimulation seen so often in acute animal experiments.

Studies on the Course of Disease. An example of our studies of this kind is given by the records, in Figure 4, of A.D., a young girl with rheumatic heart disease and mitral stenosis but without a murmur suggesting regurgitation. Admitted in her first attack of congestive failure, she had the subnormal cardiac output so often found in this condition. After 5 days of rest in bed without digitalis, diuresis occurred, the venous pressure fell and clinical improvement was marked without any corresponding improvement in the circulation. Indeed, when the venous pressure and weight had reached constant values the circulation was smaller than before.

The administration of digitalis resulted in a stimulation of cardiac output per minute before much of the drug had been given. The next day the patient went into auricular flutter which was not controlled by the small amount of digitalis then in the body; and the tachycardia increased the circulation to normal without any noteworthy change in the patient's symptoms. Continued administration of digitalis brought the ventricular rate under control 3 days later, the circulation becoming subnormal once more. After 6 days in flutter the rhythm changed to auricular fibrillation without any noteworthy change in ventricular function or cardiac output, as judged by the ballistic record. Three days later the patient complained of nausea and the ballistocardiogram record showed

the long pauses and flat diastole usually found when dosage of digitalis is excessive. It is to be noted that the slow rate carried the circulation far subnormal without any return of the congestive symptoms. Digitalis was withdrawn and improvement followed promptly.

Inspection of the records of Figure 4 draws attention to the similarity in the form and size of the systolic complexes throughout

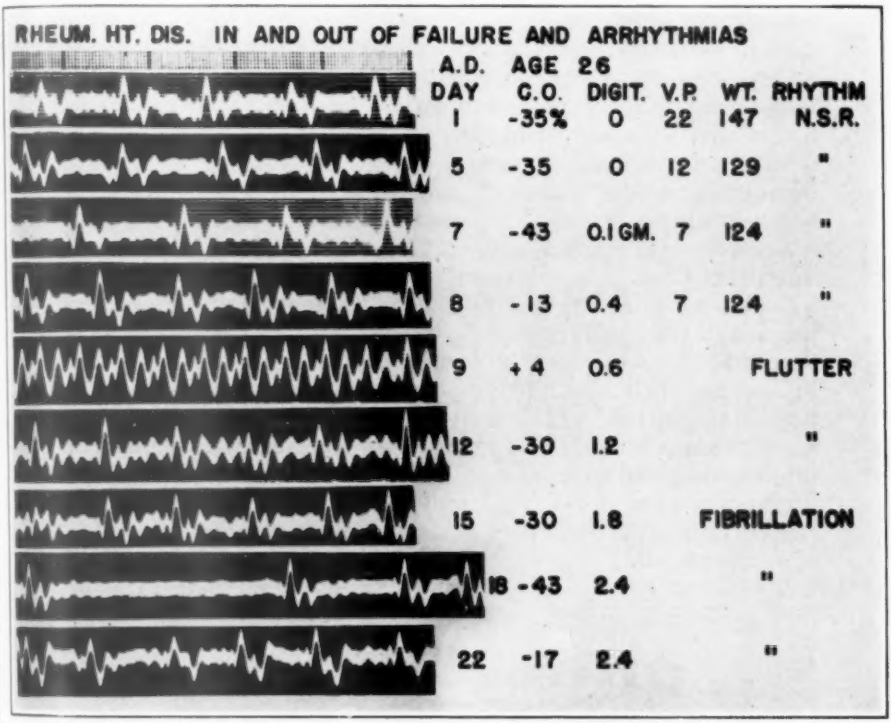
RHEUM. HT. DIS. IN AND OUT OF FAILURE AND ARRHYTHMIAS
A.D. AGE 26
DAY C.O. DIGIT. V.P. WT. RHYTHM
1 -35% 0 22 147 N.S.R.
5 -35 0 12 129 "
7 -43 0.1 GM. 7 124 "
8 -13 0.4 7 124 "
9 +4 0.6 FLUTTER
12 -30 1.2 "
15 -30 1.8 FIBRILLATION
18 -43 2.4 "
22 -17 2.4 "

FIG. 4.—Records of A.D. Female. Age 26, height 5 ft. 2 in., weight 122 pounds. Rheumatic heart disease, mitral stenosis. Marked cardiac enlargement, orthodiagram +60% of expected normal. Blood pressure varied little from 100/70 except during rapid flutter (Row 5) when it was 80/65. Digitalis is given as the total dose; the patient had not received any before admission. The cardiac output is given in per cent of the normal average; the normal limits ±22%.

the changing clinical picture. The stroke volume is quite constant and changes in the cardiac output per minute are almost entirely due to changes in rate of beating. Perhaps the cardiac disease has set a sharp limit on the stroke volume, and the patient's circulatory adaptations are largely limited to changes of rate. I have published the records of another case of rheumatic heart disease with mitral stenosis where the patient failed to show the normal increase in stroke volume after exercise.[6a]

Another impressive fact is the magnitude of the changes of cardiac output per minute without any corresponding fluctuations in the patient's sensations or general condition. I could cite many other such instances.

The Ballistocardiogram in Acute Experiments. No exposition of this instrument would be complete without mentioning its utility in acute experiments. The ease and rapidity with which records can be secured, the fact that a continuous record can be obtained if desired, the additional fact that observation of the flickering light spot gives the operator a general knowledge of the course of events which permits proper timing of the photographs, combine to put the ballistocardiographic method in a class by itself for acute experiments on the human circulation. Records showing the effects of drug action,[9] of artificial fever,[6b] of exercise,[6a] and of holding the breath[6a] have been published, and we are still actively pursuing this field whenever clinical opportunity presents itself.

Records from another acute experiment are given in Figure 2. The patient had an arteriovenous fistula of the right leg secondary to a stab wound. The records were taken before and during compression of the abnormal limb by a blood pressure cuff until pulsation in the arteries was abolished. There was marked diminution in the circulation when the escape of blood through the fistula was prevented, as has been found repeatedly, in both clinical and experimental studies (for literature see Burwell *et al.*) In our subject additional records taken after release of the cuff and during similar occlusion of the circulation in the normal leg were almost identical with that obtained before occlusion, so they have not been reproduced.

LEGEND FOR FIG. 5.

FIG. 5.—S.L. Female. Age 48, height 4 ft. 11 in., weight 119 pounds. Severe angina pectoris. Records two-thirds actual size.

Top Row. Jan. 16, 1940. Soon after admission. Blood pressure 126/80. Cardiac output −52% (normal limits ±22%).

Row 2. Jan. 22, 1940. Blood pressure 140/70. After block of left 2, 3, 4, 5 sympathetic ganglia without relief. Cardiac output −57%.

Row 3. Mar. 11, 1940. Ten days after release of common duct adhesions. Drain in common duct. Cardiac output −17%. Now within normal limits.

Rows 4 to 7. Concerned with the injection of saline solution down common duct.

Row 4. Time: 11.19. Before injection. Blood pressure 120/80. Duct pressure 150 mm. H_2O. Patient comfortable. Has had morphine, gr. $\frac{1}{6}$ s.c., recently. Cardiac output −22%.

Row 5. Time: 11.29. Fluid running in under 200 mm. H_2O head of pressure. Blood pressure 120/76. Cardiac output −43%. No pain yet. Note reduction of impacts.

Row 6. Time: 11.41. Blood pressure 130/80. Head of perfusion pressure 500 mm. Cardiac output −22% or higher. Squeezing precordial pain radiating down left arm. Perfusion stopped after this record. Note abnormal record form, the doubled point of the first downward deflection, the "double I" type.

Row 7. Time: 12.08. Blood pressure 120/80. Duct pressure 160. Cardiac output −30%. Patient comfortable. Form of record normal again.

Similar experiments have been performed on 2 other cases of traumatic arteriovenous communications. The effect of occluding

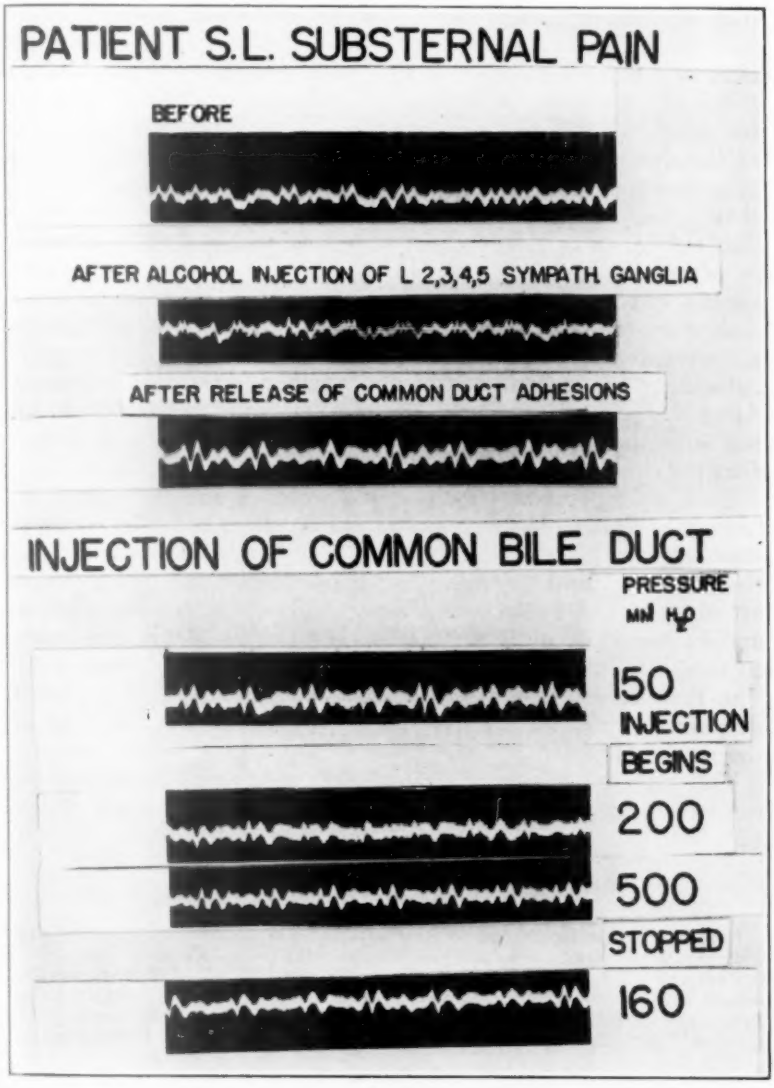

FIG. 5.

the communication has varied in the different cases and the experiment may well provide a convenient measure of the size of the abnormality.

Another acute experiment, which gave a most unexpected result, was performed with the help of Dr. H. P. Royster.[8] Patient S.L. (see Fig. 5) had been bedridden for many months with attacks of pain resembling angina pectoris, an attack being brought on by 2 trips over Dr. Master's[5] steps. At first the diagnosis had not been clear and 6 years before this admission the patient's gall bladder had been removed in the hope that this was the origin of the attacks. No relief had followed and since that time numerous operations on the sympathetic nerves had been attempted to relieve the intolerable situation. On admission to the hospital the record in Figure 5, Row 1, was secured, which indicated that the patient had a circulation only about one-half the expected normal, a finding common in severe coronary disease. Alcohol injected into sympathetic ganglia did not help; after this procedure the circulation was a little worse than before (Fig. 5, Row 2). It was to relieve a desperate situation that Dr. Ravdin attempted further common duct surgery. Adhesions were found, the common duct was freed and drained. After this operation the patient was very much better, although not entirely relieved of her attacks, and we were amazed to find that the circulation had greatly improved (Fig. 5, Row 3).

Two of Dr. Ravdin's assistants, Drs. Royster and Sanders, in pursuit of their interest in ascertaining the pressure in the common duct sufficient to open the sphincter, injected saline solution down the patient's drainage tube. To their surprise they produced an attack similar to those from which the patient had been suffering, and described as typical angina pectoris. They needed no persuasion to repeat the injection on the ballistic table (Fig. 5, Rows 4–7). The procedure not only caused pain but also marked transient depression of the circulation and an abnormality in the form of the impacts.

The pain the patient described was typical of angina pectoris but we do not wish to place too much emphasis on having caused

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LEGEND FOR FIG. 6.

FIG. 6.—Changes in the form of the ballistic record induced by operative procedures in patients with abnormal hearts. Reproduced one-half actual size.

Rows 1 to 4. L.K. Malignant hypertension. Row 1, a normal form; cardiac output per minute −27% (subnormal); Row 2, note M-shaped records. Cardiac output over −22%. Row 3, diaphragmatic impacts = D, and ejection marked. Cardiac output −35%. Row 4, note recovery of normal form. Cardiac output −17% (normal).

Rows 5 to 7. G.T. Malignant hypertension. Row 5, normal form. Cardiac output +22% (normal). Row 6, form very abnormal. Cardiac output doubtful. Row 7, form has greatly improved. Cardiac output −13% (normal).

Rows 8 to 10. K.T. Angina pectoris and calculous cholecystitis. Row 8, normal form. Cardiac output −39% (very subnormal). Row 9, note change to abnormal form. Cardiac output uncertain. Row 10, return to normal form. Cardiac output −43%.

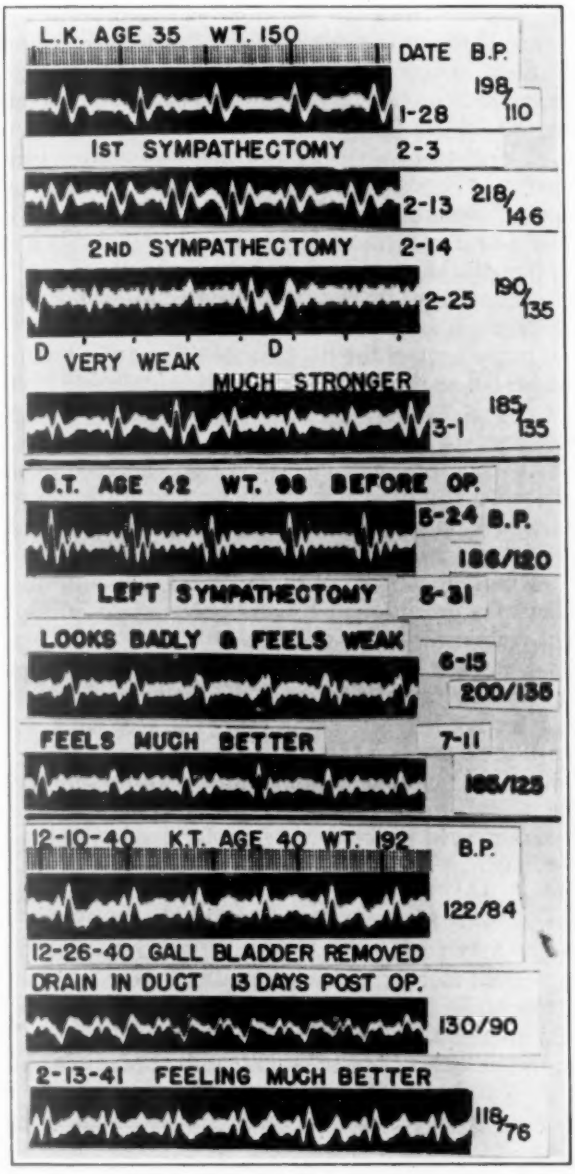
L.K. AGE 35 WT. 150
DATE B.P.
198/110
1-28
1ST SYMPATHECTOMY 2-3
2-13 218/146
2ND SYMPATHECTOMY 2-14
2-25 190/135
D VERY WEAK D
MUCH STRONGER
3-1 185/135
G.T. AGE 42 WT. 98 BEFORE OP.
5-24 B.P.
186/120
LEFT SYMPATHECTOMY 5-31
LOOKS BADLY & FEELS WEAK
6-15
200/135
FEELS MUCH BETTER 7-11
165/125
12-10-40 K.T. AGE 40 WT. 192
B.P.
122/84
12-26-40 GALL BLADDER REMOVED
DRAIN IN DUCT 13 DAYS POST OP.
130/90
2-13-41 FEELING MUCH BETTER
118/76

FIG. 6.

angina by distending the bile duct. The patient had suffered many things from many physicians. She had the suggestibility of the chronic invalid and she knew the story expected perfectly. But in the difference between the records there is an objective demonstration that there is something operating from the biliary passages which can depress the general circulation profoundly. This provides a definite physiologic background for the vague relation between hepatic and cardiac disease which is so often recognized by clinicians.

Changes in the Form of the Ballistic Record. The majority of the data presented before might have been attained by other cardiac output methods if a team of highly trained operators had worked for a long enough time. In such cases the advantage of the ballistocardiograph consists of its ease and rapidity of operation. But the ballistocardiograph may also demonstrate cardiac abnormality by alterations in the form of the ballistic record and so provide evidence of cardiac health or disease of a type not obtainable by any other method.

Abnormalities of the form of the complexes are common in persons with collateral evidence of serious heart disease, and, if present, they usually persist in spite of therapeutic measures. The records of 3 cases in which the distortion was temporary are shown in Figure 6. Each of these patients exhibited some collateral evidence of cardiac abnormality on admission and the ballistic abnormality was precipitated by an operation and disappeared as the patient recovered from its effects.

G.T. had essential hypertension and an enlarged heart. The cardiac output on admission and the ballistic form were both normal. After her first sympathectomy she recovered slowly from the effects of the operation and she looked badly and felt weak when the second record was taken. Compared with the previous record, the initial downward deflection, *I* wave, is shallower and more rounded; the upward peak, *J* wave, is flattened or split and much reduced in height. The time between the second downward deflection, *K* wave, and the beginning of ejection is prolonged. This is the form the ballistic record is expected to take when the ejected blood attains maximum velocity late rather than early in systole.

As this patient recovered, the form of the record returned to the normal. She underwent a second sympathectomy successfully and no ballistic abnormality was detected after it.

L.K., a second case of hypertension, had a heart whose silhouette area was 21% above the expected normal and a subnormal cardiac output per minute. After the first sympathectomy the records became M-shaped. After the second operation she was very weak; diaphragmatic impacts showed the labored breathing and the cardiac impacts were greatly reduced in amplitude. As she recovered, the ballistic form returned to normal. The changes of ballistic form

seen in this case are quite like some of those demonstrated in an acute experiment on an anesthetized dog subjected to asphyxia.[11]

K.T. had angina pectoris and exhibited the subnormal circulation found so frequently in these cases. After removal of the gall bladder the ballistic form changed profoundly, the prominent upward deflection (*J* wave) being greatly reduced in size and the second downward deflection (*K* wave) moving far back in systole. As the patient recovered her strength the ballistic form returned to normal.

Records obtained on persons with normal hearts subjected to operations have not shown any ballistic abnormality during the postoperative period, but I have only a few data on the point.

Summary. This presentation is designed to demonstrate the utility of the ballistocardiogram as an instrument for the diagnosis of cardiac and circulatory abnormality and for the solution of certain clinical problems. Records are presented indicating changes in the circulation in congestive heart failure and on recovery from it, during digitalis action, and in various arrhythmias in one patient.

The method is especially suited for acute clinical experiments. The examples cited include ballistocardiograms from a case of traumatic arteriovenous communication before and during occlusion of the communication, and similar records from a patient with angina pectoris in whom pain was produced by the injection of fluid down a drainage tube into the common bile duct.

Changes in the form of the ballistic record provide evidence of cardiac dysfunction of a type not obtainable by other methods. The examples cited were obtained from 3 patients in whom the abnormality followed operative procedures, and disappeared as strength was regained.

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SYNOVIAL FLUID AND SYNOVIAL MEMBRANE ABNORMALITIES RESULTING FROM VARYING GRADES OF SYSTEMIC INFECTION AND EDEMA.*†

By Howard C. Coggeshall, M.D.,
FORMER JOHN WHITE BROWNE SCHOLAR, HARVARD MEDICAL SCHOOL,

Granville A. Bennett, M.D.,
ASSISTANT PROFESSOR IN MEDICINE, HARVARD MEDICAL SCHOOL,

Charles F. Warren, M.D.,
FORMER DALTON SCHOLAR, MASSACHUSETTS GENERAL HOSPITAL,
AND
Walter Bauer, M.D.,
ASSOCIATE PROFESSOR IN MEDICINE, HARVARD MEDICAL SCHOOL; PHYSICIAN IN MEDICINE, MASSACHUSETTS GENERAL HOSPITAL,
BOSTON, MASS.

(From the Medical Clinic of the Massachusetts General Hospital, the Departments of Medicine and Pathology, Harvard Medical School, and the Massachusetts Department of Public Health.)

For a number of years, this laboratory has been concerned with studies pertaining to the physiology of normal joints and the alterations produced by disease.‡[1,3,8–10] The present report, a continuation of previous work,[3] was undertaken to correlate various degrees of systemic infection and subcutaneous edema occurring either separately or combined with abnormalities of synovial fluid and membrane.

Materials and Methods. Synovial fluid was aspirated as completely as possible from 156 knee joints of 103 postmortem cases and examined within 30 minutes of death. The techniques employed have been described.[3] Long strips of tissue, including synovial lining and subsynovial tissue, were removed from the pouches lateral and medial to the patellæ of 49 joints. These specimens were fixed in Zenker's fluid and in 10% formalin. Representative blocks of tissue, including the external part of the capsule, were embedded in celloidin or paraffin. The cut sections were stained with eosin and methylene blue or hematoxylin and eosin. The degree and extent of edema were known from antemortem examination. The type, severity and duration of the infectious process were estimated on the basis of complete clinical and laboratory records and postmortem studies. The latter were complete in 53 instances. Patients having died from infection without demonstrable edema were divided into 3 groups according to the severity of the infection. Those with non-infectious diseases, evincing edema, were classified separately. When both infection and edema were present, the cases were grouped according to the severity of the infection. Terminal infections, sharply localized to one organ or system, complicating such disorders as carcinomatosis, cerebral hemorrhage, asthma, and so on, were designated as minimal. When the infection was more widespread, especially if bacterial metastases had occurred, it was classified as moderate.

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The group with severe infection comprised cases with proved or presumptive septicemia. This classification is admittedly arbitrary and may have been based in some instances on an erroneous interpretation but offers an approximate and for the present purposes sufficient indication of the extent of the infectious process.

Accordingly, the 156 fluids were segregated into the following groups:

I. 37 from cases with minimal infection without edema
II. 24 from cases with moderate infection without edema
III. 20 from cases with severe infection without edema
IV. 18 from cases with edema, without infection
V. 27 from cases with edema and minimal infection
VI. 30 from cases with edema and moderate or severe infection.

Results. *Synovial Fluid Abnormalities.* It is evident from Table 1 that systemic infection and edema produce characteristic deviations from normal in the synovial fluid. The normal values used for comparison are taken from previously published data.[3]

TABLE 1.—VARIATIONS IN THE AMOUNT AND CELLULAR COMPOSITION OF SYNOVIAL FLUID RESULTING FROM VARYING GRADES OF INFECTION AND EDEMA.

Group.	Total No. of joints examined.	Clinical data.		Amt. of synovial fluid, (cc.).	Nucleated cells in synovial fluid.								Synovial and unclassified cells.		Variations observed.
		Peripheral edema.	Degree of infection.		No. per c.mm.	Neutrophils.		Macrophages.		Lymphocytes.					
						%	Absolute.	%	Absolute.	%	Absolute.		%	Absolute.	
Normal	29	Absent	Absent	0.13	13	0.0	0.0	0.0	0.0	6.0	1.7		0.0	0.0	Minimum
				2.00	180	25.0	18.0	83.0	106.0	78.0	61.2		18.0	9.6	Maximum
				0.45	63.2	6.5	3.3	62.9	41.7	24.6	14.7		5.6	3.5	Average
I	37	Absent	Mild	0.13	4	0.0	0.0	20.0	5.3	0.0	0.0		0.0	0.0	Minimum
				7.50	400	60.0	342.0	98.0	315.0	72.0	176.0		10.0	45.0	Maximum
				1.20	152	17.9	37.7	55.4	74.3	26.5	33.1		2.9	4.0	Average
II	24	Absent	Mod.	0.10	20	0.0	0.0	6.0	12.0	0.0	0.0		0.0	0.0	Minimum
				4.50	9,500	94.0	6,650.0	84.0	2280.0	88.0	1003.2		20.0	44.4	Maximum
				0.74	656	29.0	379.9	40.5	166.9	26.3	102.7		4.1	6.7	Average
III	20	Absent	Severe	0.13	30	0.0	0.0	9.0	10.2	0.0	0.0		0.0	0.0	Minimum
				8.00	8,850	90.0	7,965.0	93.0	796.5	42.0	542.0		8.0	97.5	Maximum
				1.40	1,022	34.5	612.2	49.0	252.0	16.5	150.8		1.4	7.8	Average
IV	18*	Present	Absent	2.00	1	0.0	0.0	4.0	1.0	0.0	0.0		0.0	0.0	Minimum
				40.00	128	98.0	49.0	80.0	35.9	68.0	81.9		10.0	8.0	Maximum
				10.70	32	22.1	9.2	51.9	13.6	23.9	12.8		1.6	1.7	Average
V	27*	Present	Mild	0.40	6	0.0	12.0	4.4	0.0	4.0	0.6		0.0	0.0	Minimum
				30.00	665	46.0	88.0	206.1	199.5	80.0	432.3		12.0	20.0	Maximum
				5.30	75	9.4	6.5	57.4	37.4	30.2	33.7		2.0	2.3	Average
VI	30	Present	Mod. and severe	0.30	13	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	Minimum
				20.00	24,700	96.0	23,218.0	89.0	3159.0	84.0	390.0		12.0	183.6	Maximum
				5.10	2,059	38.5	1,516.4	44.5	352.5	13.0	65.6		2.3	11.6	Average

* Two synovial fluids in Groups IV and V did not have differential cell counts.

Amount of Synovial Fluid. The amount of synovial fluid aspirated from the 156 knee joints showed individual variations from 0.1 to 40 cc. as compared to minimal-maximal variations of 0.13 to 2.0 cc. and an average value of 0.45 cc. for normal adult knee joints (see Table 1 and Chart 1.)

From the figures below, it is apparent that increased amounts of synovial fluid were usually found in patients with edema, the common causes of which were cardiac failure, nephritis, lowered serum proteins, phlebitis and regional hypodermoclyses.

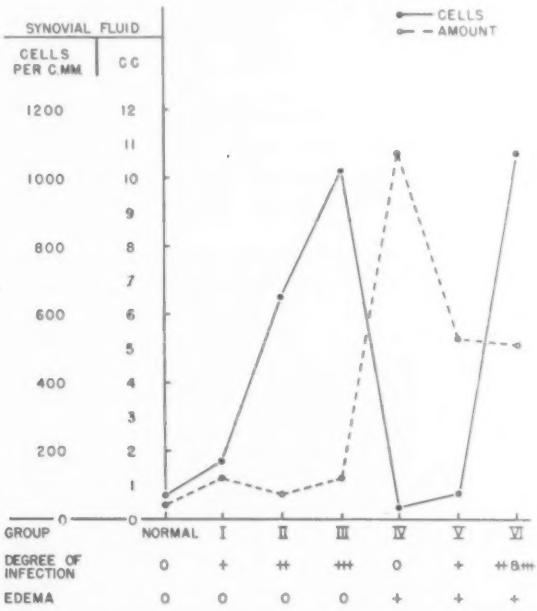

CHART 1.—The average amount of synovial fluid obtained from the knee joints of normal individuals and individuals dying with varying grades of infection and edema. The average total nucleated cell counts are likewise presented.

Synovial Fluids Obtained from Edematous Patients. Of 75 specimens, 25 were less than 2 cc. in amount, 15 were between 2 and 5 cc., and 35 were more than 5 cc. The majority of these fluids had a normal cytology. Of 50 specimens, each amounting to more than 2 cc., from patients with recognizable edema, 11 showed an increased number of nucleated cells, and 14 had an elevated neutrophil count. Of 12 specimens surpassing the normal quantity, 7 were derived from patients without demonstrable edema but minimal infection and showed normal cytology, whereas 5 from non-edematous individuals with some infections proved to have increased cell counts. The largest effusions, 20 to 40 cc., were aspirated from patients with cardiac edema. In such cases, the fluid content of the two knees

was practically the same. These findings suggest that the physiologic alterations[5] responsible for the various types of edema resulted in an increased transudation into the joints. That such increased amounts of synovial fluid consisted largely of plasma water is attested to by low nucleated cell counts, reduced viscosities and lowered content of total solids, protein and mucin.[9]

The amount of fluid obtained from patients dying with infection was generally less than 2 cc. unless edema was present. Of 81 synovial fluids obtained from patients with infection but without edema, 69 were 2 cc. or less, 12 were more than 2 cc. Most of these

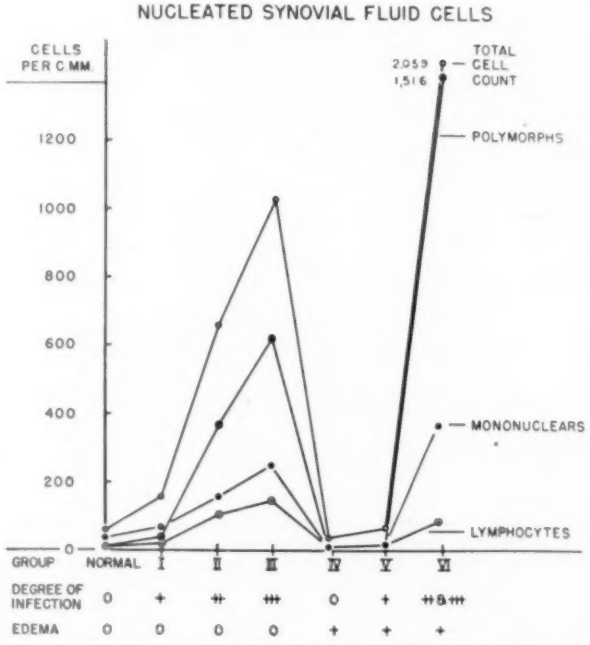

CHART 2.—The average total nucleated cell, neutrophil, mononuclear and lymphocyte counts of normal human synovial fluid and of synovial fluids obtained from patients dying with varying grades of infection and edema.

specimens showed cytologic abnormalities. Increased amounts of synovial fluid were present in 20% (4 fluids) of 11 patients with severe infection alone, but in 70% (21 fluids) of 17 individuals who showed both moderate or severe infection and edema. These observations indicate that although inflammation of the synovial tissues was frequently present in patients with infection, usually it was not of sufficient grade to permit increased transudation into the joints.

Total Number of Nucleated Cells and Individual Cell Types. The total nucleated cell counts of normal human fluid specimens range

from 13 to 180 with an average of 63 per c.mm. (Table 1.) The cell types are expressed as percentage figures and absolute counts, since the latter method portrays more accurately the cellular constituents. The percentages and absolute numbers of cells per c.mm. in normal fluid are: 62.9% or 41.7 macrophages, 24.6% or 14.7 lymphocytes, 6.5% or 3.3 neutrophils, 4.3% or 2.5 synovial cells and 2.2% or 0.8 unidentified cells. Neutrophils range from 0 to 25%, the absolute counts from 0 to 18. In the analysis of the present data, a total nucleated cell count of over 180 and an absolute neutrophil count above 18 are considered normal.

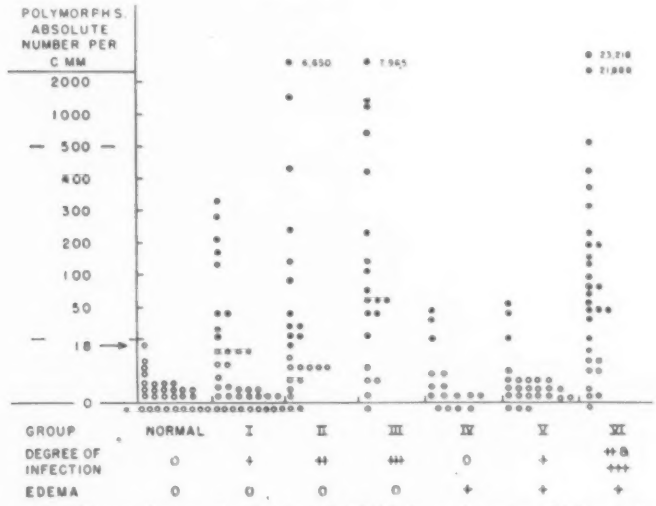

CHART 3.—Shows the number of synovial fluids in each group which contained an increased number of neutrophils, the established normal being 18 per c.mm. The values listed below the base line (*) represent fluids that contained no neutrophils. Only 16 of the 18 fluids in Group IV and 25 of the 27 fluids in Group V had differential cell counts; therefore only 181 dots appear on this chart.

As can be seen from Table 1, the total nucleated cell counts in the present series varied from 1 to 24,700. The highest counts were observed in cases with both severe infection and edema (Group VI), the next highest in the fluids obtained from patients with severe infections but no edema (Group III), and the lowest in the group showing edema only (Group IV). Chart 2 shows that the elevated total cell counts were due chiefly to increases in the neutrophils. The highest neutrophil count, 23,218 (84%), was found in a fluid having a total cell count of 24,700 (Case 24, Table 2). This specimen was obtained from a patient dying from a ruptured appendix and a complicating hemolytic streptococcus septicemia. The number of fluids in each group that had an increased number of neutrophils is readily seen in Chart 3.

ANALYSIS OF TOTAL NUCLEATED AND NEUTROPHIL COUNTS.

Number of fluids.	Type of case.		Number with increased total nucleated cell counts.	Number with increased neutrophils.
	Infection.	Edema.		
81	+	0	30	36
57	+	+	21	24
18	0	+	0	3

From the above table it will be apparent that 63 of the 156 fluids had increased absolute neutrophil counts as compared to 51 with elevated total nucleated cell counts.

FIG. 1.—Normal synovial lining tissue (from the right knee of Case 8, Table 2, Group I). (Orig. mag. × 310.) This patient died of thyrotoxicosis, diabetes mellitus and bronchopneumonia. The synovial fluids obtained from the knees of this patient were normal. (Section stained with hematoxylin-eosin.) (Small numbers of leukocytes are usually found if sufficiently large areas of the synovial membrane and subsynovial tissues are examined. The presence of 1 to 3 leukocytes per field (× 900) was not considered abnormal unless found in most of the fields examined, or accompanied by perivascular accumulations in the subsynovial tissue.)

Correlations of Synovial Fluid and Synovial Membrane Abnormalities. In order to obtain further information concerning the significance of the synovial fluid abnormalities, synovial tissue specimens were examined microscopically and the degree of cellular infiltration in representative oil immersion fields (× 900) was recorded. Note was made of the approximate number of neutrophils, mononuclears, lymphocytes, plasma cells and the presence or absence of perivascular inflammatory cell accumulations. The degree of inflammation present was recorded as none, minimal, moderate and severe. The synovial fluid findings, the clinical data and histologic studies presented in Table 2 were not compared with one another until the microscopic sections had been described.

TABLE 2.—POSTMORTEM OBSERVATIONS OF THE SYNOVIAL FLUID CYTOLOGY AND SYNOVIAL MEMBRANE FROM INDIVIDUALS WITH VARYING DEGREES OF INFECTION.

Case No.	Group No.	Peripheral edema.	Degree infection.	Knee.	Amt. of synovial fluid (cc.).	Nucleated cells in synovial fluid.										Synovial membranes.		Blood leukocyte count (in thous.).	Postmortem cultures.		Anatomic diagnoses.
						Total No. per cu. mm.	Neutrophils.		Macrophages.		Lymphocytes.		Synovial and unclassified cells.			Normal.	Abnormal.		Synovial fluid.	Blood.	
							%	Absolute.	%	Absolute.	%	Absolute.	%	Absolute.							
1	Normal	0	0	R	0.20	20	25	5.0	0	0.0	65	13.0	10	2.0		+	..	Not done	No growth	Not done	Chr. hem. 5½ hr. before death.
				L	0.50	50	20	16.0	10	5.0	60	30.0	10	5.0		+	..	Not done	No growth	Not done	
2	I	0	+	R	7.00	4	0	0.0	66	2.6	34	1.4	0	0.0		+	..	13.8	Not done	Not done	Bronchogenic carc. with gen. metastasis; chr. pneu., rt. up. lobe.
3	I	0	+	R	0.13	20	10	2.0	80	16.0	10	2.0	0	0.0		+		10.0	Not done	Not done	Carc. of stom. with perf.; subphrenic abscess; peritonitis; bilat. br.pneu.
				L	0.13	30	10	3.0	60	18.0	30	9.0	0	0.0		+		10.0	Not done	Not done	
4	I	0	+	R	2.50	40	40	16.0	0	0.0	60	24.0	0	0.0		+	..	16.7	Not done	Not done	Bronch. asthma with asphyxia; ac. mucopur. bronchitis; chr. pleuritis; pericarditis.
				L	5.00	30	50	15.0	0	0.0	50	15.0	0	0.0		+	..	16.7	Not done	Not done	
5	I	0	+	R	0.50	315	0	0.0	100	315.0	0	0.0	0	0.0		+	..	16.0	Not done	Staph. aureus	Dissect. aneurysm of aorta with rupture into pericardium; ac. central necrosis of liver.
				L	0.30	185	2	3.7	98	181.3	0	0.0	0	0.0		+	..	16.0	Not done	Staph. aureus	
6	I	0	+	R	0.50	60	0	0.0	56	33.6	40	24.0	4	2.4		+	..	33.6	Not done	B. coli	Retroperitoneal abscess (B. coli); bronchopneu.
				L	0.50	50	4	2.0	72	36.0	20	10.0	4	2.0		+	..	33.6	Not done	B. coli	
7	I	0	+	R	0.50	400	30	120.0	26	104.0	44	176.0	0	0.0		+	..	15.4	No growth	Not done	Epidermoid carc. of lung; bronchopneu.; chr. pleuritis.
				L	0.50	400	40	160.0	20	80.0	40	160.0	0	0.0		+	..	15.4	No growth	Not done	
8	I	0	+	R	0.20	80	56	45.0	24	19.0	20	16.0	0	0.0		+	..	16.0	No growth	Not done	Bronchopneu.; diabetes mellitus; thyrotoxicosis.
				L	0.20	10	40	4.0	40	4.0	20	2.0	0	0.0		+	..	16.0	No growth	Not done	
9	I	0	+	R	0.50	50	50	25.0	25	12.5	25	12.5	0	0.0		..	+	13.4	Not done	B. coli	Pemphigus.
				L	5.00	30	25	7.5	0	0.0	75	22.5	0	0.0		..	+	13.4	Not done	B. coli	
10	II	0	++	R	0.50	60	40	24.0	48	28.8	10	6.0	2	1.2		+	..	9.0	Not done	No growth	Perforated appendix; gen. peritonitis; bronchopneu.
				L	0.50	35	30	10.5	53	18.5	9	3.0	8	3.0		+	..	9.0	Not done	No growth	
11	II	0	++	R	0.30	85	2	1.7	82	69.7	10	8.5	6	5.1		+	..	19.0	Not done	No growth	Ac. intestinal obst.; peritonitis with multiple absc.; bronchopneu.; bilateral pleuritis; esoph. ulceration.
				L	2.00	55	8	4.4	82	45.1	4	2.2	6	3.3		+	..	19.0	Not done	No growth	
12	II	0	++	R	1.00	920	44	404.8	22	202.4	34	312.8	0	0.0		..	+	18.0	Not done	No growth	Bronchopneu.; pulm. infarct.; ac.
				L	1.00	130	14	18.2	62	80.6	22	28.6	2	2.6		..	+	18.0	Not done	No growth	bronchitis; carbon monoxide poi-

11	II	0	++	R	0.30	85	2	1.7	82	69.7	10	8.5	6	5.1	+	..	19.0	Not done	No growth	Ac. intestinal obst.; peritonitis with multiple absc.; bronchopneu.; bilateral pleuritis; esoph. ulceration.
				L	2.00	55	8	4.4	82	45.1	4	2.2	6	3.3	+	..	19.0	Not done	No growth	
12	II	0	++	R	1.00	920	44	404.8	22	202.4	34	312.8	0	0.0	..	+	18.0	Not done	No growth	Bronchopneu.; pulm. infarct.; ac. bronchitis; carbon monoxide poisoning; decubitus ulcers.
				L	1.00	130	14	18.2	62	80.6	22	28.6	2	2.6	..	+	18.0	Not done	No growth	
13	II	0	++	R	0.50	9,500	70	6,650.0	24	2280.0	6	570.0	0	0.0	..	+	15.0	Not done	No growth	Bilat. lobar pneu. (Type IV); bilat. ac. fibrinous pleuritis.
				L	0.50	1,400	94	1,316.0	6	84.0	0	0.0	0	0.0	..	+	15.0	Not done	No growth	
14	III	0	+++	R	1.00	80	2	1.6	75	60.0	23	18.4	0	0.0	+	..	15.0	Not done	Hem. strep.	Peritonitis (post-cholecystectomy).
				L	0.75	100	10	10.0	70	70.0	20	20.0	0	0.0	+	..	15.0	Not done	Hem. strep.	
15	III	0	+++	R	0.75	400	8	32.0	57	228.0	35	140.0	0	0.0	..	+	26.2	Not done	Pneu. Type III	Pneumoc. (Type III) meningitis; septicemia; otitis media; bronchopneu. and endocarditis.
				L	0.75	80	0	0.0	94	75.2	6	4.8	0	0.0	..	+	26.2	Not done	Pneu. Type III	
16	III	0	+++	R	0.75	830	25	207.5	38	315.4	34	282.2	3	24.9	..	+	9.0	Not done	No growth	Subac. bact. endocarditis; rheu. heart dis.; infarcts of kidney, spleen and brain.
				L	1.00	1,625	26	422.0	36	585.0	32	520.0	6	98.0	..	+	9.0	Not done	No growth	
17	IV	+	0	R	3.00	128	8	10.2	28	35.8	64	82.0	0	0.0	..	+	10.0	Not done	No growth	Chr. glom. nephritis; hypertensive heart dis.; ascites; hydrothorax.
				L	7.00	48	7	3.4	43	20.6	50	24.0	0	0.0	+	..	10.0	Not done	No growth	
18	V	+	+	R	0.80	50	6	3.0	64	32.0	28	14.0	2	1.0	+	..	9.0	Not done	No growth	Hypertensive heart dis.; pulm. infarct.; bilat. bronchopneu.; hydrothorax; hydropericardium.
				L	0.80	50	6	3.0	49	24.5	39	19.5	6	3.0	+	..	9.0	Not done	No growth	
19	V	+	+	R	3.00	90	6	5.4	32	28.8	58	52.2	4	3.6	+	..	10.0	Not done	No growth	Pemphigus; thrombosis, left renal vein; infarct, left adrenal.
				L	2.00	190	16	30.4	66	125.4	18	34.2	0	0.0	+	..	10.0	Not done	No growth	
20	V	+	+	R	10.00	110	75	82.5	0	0.0	25	27.5	0	0.0	..	+	10.0	Not done	No growth	Chr. glom. nephritis; rheu. heart dis.; bilat. hydrothorax and ascites.
				L	13.00	70	50	35.0	50	35.0	0	0.0	0	0.0	..	+	10.0	Not done	No growth	
21	VI	+	++	R	7.00	115	54	62.1	46	52.9	0	0.0	0	0.0	+	..	5.5	Not done	No growth	Ac. bact. endocarditis; lung absc.; infarcts, spleen and kidneys; adenocarc. of gall bladder.
				L	3.50	345	68	234.6	31	106.9	0	0.0	1	3.5	+	..	5.5	Not done	No growth	
22	VI	+	+++	R	2.50	220	64	140.8	34	74.8	0	0.0	2	4.4	+	..	18.0	Not done	Hem. strep.	Hem. strep. septicemia; phlebitis rt. saphen. vein; pulm. and embolic absc.; ac. fibrinous pleuritis; ureteritis (rt.).
				L	1.50	385	48	184.8	38	146.3	10	38.5	4	15.4	+	..	18.0	Not done	Hem. strep.	
23	VI	+	+++	R	4.00	315	48	151.0	20	63.0	32	101.0	0	0.0	+	..	39.4	Not done	Hem. strep.	Pemphigus; chr. fibrinous pleuritis (left); pyelonephritis; hemolytic strep. septicemia.
				L	1.50	110	48	53.0	30	33.0	22	24.0	0	0.0	+	..	39.4	Not done	Hem. strep.	
24	VI	+	+++	R	16.00	22,800	96	21,888.0	4	912.0	0	0.0	0	0.0	..	+	19.4	Not done	Hem. strep.	Perforated appendix; append. absc.; peritonitis; hem. strep. septicemia; diabetes mellitus; ac. nephritis.
				L	20.00	24,700	94	23,218.0	6	1482.0	0	0.0	0	0.0	..	+	19.4	Not done	Hem. strep.	
25	VI	+	+++	R	6.00	722	51	368.0	30	216.6	17	123.0	2	14.4	..	+	7.5	Not done	No growth	Sub. bact. endocarditis.
				L	6.00	922	69	636.0	17	156.7	12	111.0	2	18.3	..	+	7.5	Not done	No growth	

Tissue specimens from both knees of 15 patients and one knee of 2 others were normal (see Table 2 and Figure 1). The tissue from the opposite knee joint of one of the latter cases (Case 17) was slightly abnormal, showing small collections of lymphocytes, plasma cells and macrophages in the synovial tissues. As can be seen from Table 2 and Chart 4—24 of the fluids obtained from these 32 joints had normal total nucleated cell counts. Two of the remaining 8 had counts of 185 and 190 and in no instance did the counts

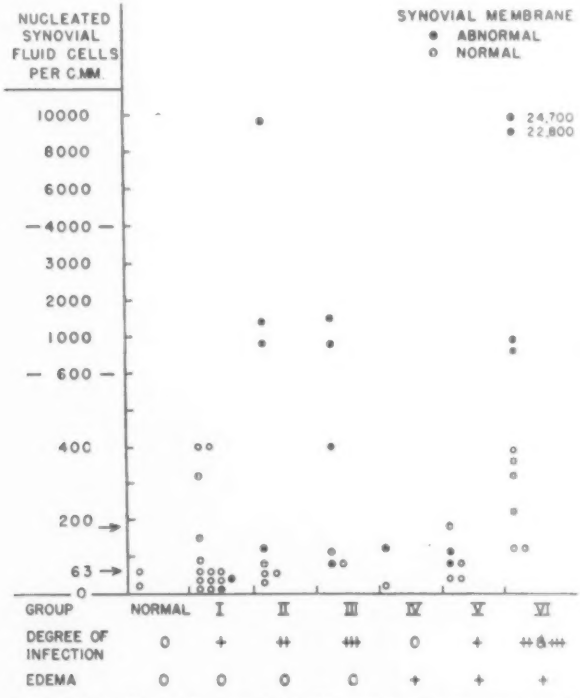

CHART 4.—Depicts the total synovial fluid nucleated cell counts of joints from which synovial tissue specimens were obtained. Normal human synovial fluids vary from 13 to 180, the average being 63 cells per c.mm. The synovial fluid and membrane abnormalities observed are readily apparent.

exceed 400. Twenty-one of these 32 fluids had normal absolute neutrophil counts (see Table 2 and Chart 5), while the remaining 11 varied from 24 to 234 neutrophils, the average being 109. Failure to remove adequate samples of synovial tissue may explain these slight discrepancies between fluid and tissue findings since often all portions are not equally involved by an inflammatory process. From these results it would appear that the synovial tissues may be expected to show little or no evidence of inflammation in those joints in which the total nucleated cell counts do not exceed 400.

Seventeen synovial tissue specimens showing microscopic evidence of inflammation were obtained from 9 (Cases 9, 12, 13, 15, 16, 17, 20, 24 and 25) individuals. The following table presents in concise form the chief histologic, clinical, and synovial fluid findings in these cases.

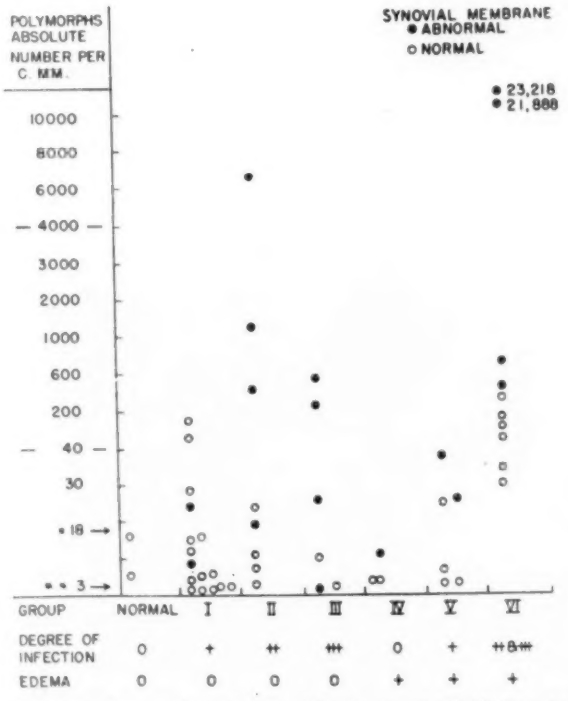

CHART 5.—Shows the number of neutrophils in synovial fluids from joints having abnormal synovial tissues. Only 3 of the synovial fluids from joints having abnormal synovial tissues had normal absolute neutrophil counts (0 to 18 per c.mm., the average being 3). It will be seen that in 11 instances the neutrophils were increased yet the synovial tissues from these same joints were normal.

TABLE 3.—CLINICAL, SYNOVIAL FLUID AND HISTOLOGIC FINDINGS IN THE CASES WITH ABNORMAL SYNOVIAL TISSUES.

Degree of synovial tissue inflammation.	No. of cases.	Grade of infection present.	Total nucleated cell counts.		Total neutrophils.	
			Normal.	Increased.	Normal.	Increased.
Minimal . . .	5	1—none 4—minimal	5	0	2	3
Moderate . . .	6	1—moderate 5—severe	2	4	2	4
Severe	6	3—moderate	0	6	0	6

Five specimens from Cases 9, 17 and 20 showed minimal inflammatory changes, consisting of small scattered perivascular accumulations of lymphocytes and plasma cells as illustrated in Figure 2. Mononuclears and neutrophils were present in small numbers.

Tables 2 and 3 reveal that such slight perivascular accumulations may be accompanied by an increase in the synovial fluid neutrophils even though the total cell counts are normal. Very slight increases in the size and number of synovial villi were present in these sections.

Six specimens obtained from cases of moderate and severe infection (Cases 12 [left knee], 13, 15 and 25 [left knee]) showed moderate inflammatory changes. Increased numbers of neutrophils, more evenly distributed than the other types of cells, were most frequent upon or within the synovial lining tissues. In addition, all types of inflammatory cells, diffusely scattered, and with less tendency to accumulate perivascularly, were present. As can be seen from

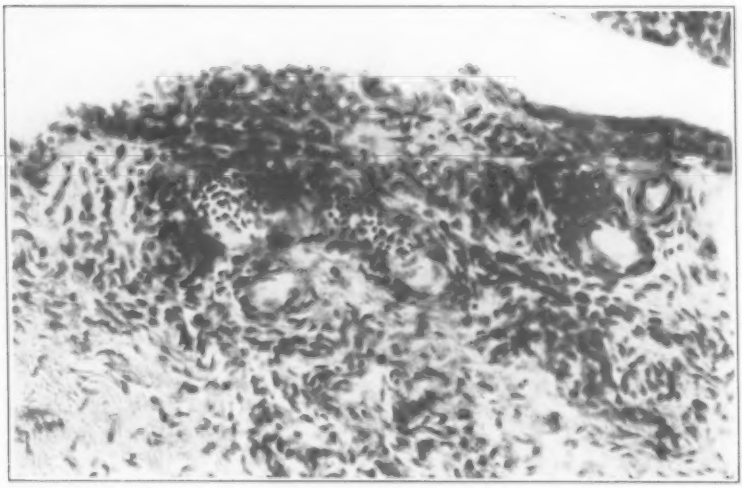

FIG. 2.—Minimal inflammatory changes seen in the synovial tissues from the right knee of Case 9 (Group II, Table 2). (Orig. mag. × 290.) Here, the small focal areas of lymphocytic infiltration are readily seen. These focal lesions presumably accounted for the slight cytologic changes observed in the fluid obtained from this joint (50 nucleated cells of which 25 were neutrophils). (Celloidin section stained with hematoxylin-eosin.)

Tables 2 and 3, two of the synovial fluids in this group had both normal total nucleated and neutrophil counts. The synovial tissues from both knees of Case 15 showed the same degree of inflammatory change, yet the fluid from only the right was abnormal.

Six other examples from cases exhibiting moderate or severe infection (Cases 12 [right knee], 16, 24 and 25 [right knee]) showed extensive inflammatory changes. The tissue specimens obtained from Case 24, dying with severe infection (perforated appendix, appendiceal abscess and hemolytic streptococcus septicemia), exhibited marked acute inflammatory reaction characterized by widespread infiltration of the synovial lining and subsynovial tissues (see Fig. 3). Neutrophils predominated but mononuclears and

lymphocytes were present. Numerous bacteria consistent with streptococci were seen. The synovial fluid nucleated cell counts in these two instances were 22,800 and 24,700 per c.mm., 96 and 94% of which were neutrophils.

The four synovial tissue specimens removed from both knees of Case 16 and from the right knees of Cases 12 and 25 showed, in addition to slight diffuse and focal perivascular cell infiltration (Fig. 4), one or more sharply defined acute inflammatory lesions (Figs. 5 and 6), with marked infiltrations of neutrophils and mononuclears, fibrin deposition, and tissue necrosis. In 2 cases thrombosis

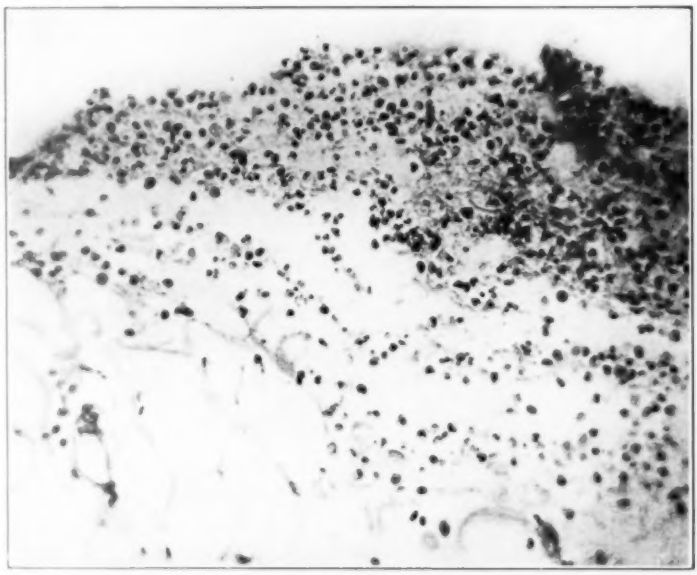

FIG. 3.—Heavy infiltration of the synovial lining and subsynovial tissues with neutrophils. (Orig. mag. × 290.) A similar degree of acute inflammation was observed in the opposite joint. The total nucleated cell counts were 22,800 and 24,700 with 96% and 94% neutrophils, respectively (Case 24, Group VI).

of arterioles was observed. One of these lesions was perivascular and deep in the subsynovial tissues (Fig. 5), the others were in the synovial lining and the adjacent subsynovial tissue (Fig. 6). These lesions were probably embolic in nature, although no microörganisms were seen. The nucleated cell counts on the fluids from these last 4 joints varied from 830 to 1625; the absolute number of neutrophils ranged from 207 to 422. Microscopic edema was seen in the tissues from Cases 24 and 25.

It is interesting that the tissues from the right knee of Case 12 showed extensive inflammatory changes, whereas the specimen from the left knee showed only moderate change. The fluid from the

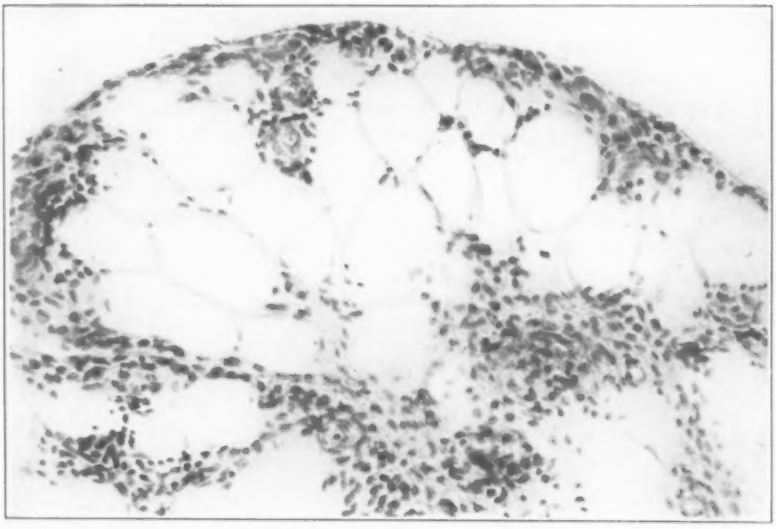

FIG. 4.—The synovial membrane over the infrapatellar fat pad of the right knee joint, showing numerous macrophages and neutrophils in perivascular areas and the synovial lining tissue. (Orig. mag. × 275.) This patient, dying of subacute bacterial endocarditis had embolic lesions in various organs. Several inflammatory lesions like the one shown in Figure 5 were observed near large arterioles deep in the subsynovial tissues (Case 16, Group III, Table 2). (Celloidon section, hematoxylin-eosin stain.)

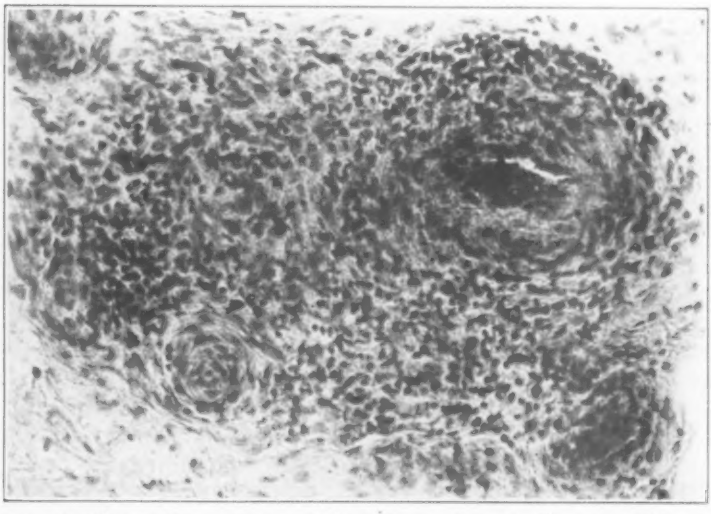

FIG. 5.—Another portion of the synovial membrane shown in Figure 4. This acute inflammatory lesion encircled a thrombosed arteriole. (Orig. mag. × 270.)

right knee had total nucleated and absolute neutrophil counts of 920 and 405 respectively, while the counts of the left were normal. Similar findings were observed in Case 25, where, however, the fluid from the knee betraying the more extensive inflammatory changes showed the less marked cytologic abnormalities.

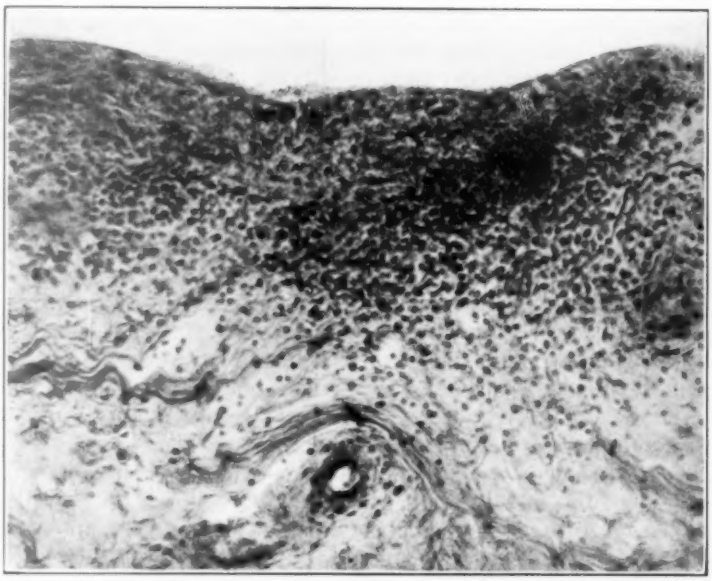

FIG. 6.—Sharply circumscribed acute inflammatory lesion in the synovial lining and subsynovial tissues from another patient dying of subacute bacterial endocarditis. (Orig. mag. × 275.) The remaining portions of the synovial membranes removed from the two knee joints revealed only slight perivascular infiltration in the subsynovial tissues. Synovial fluid studies revealed an increase in total number of nucleated cells and also in the percentage of neutrophils. (See Case 25, Group VI, Table 2.)

Discussion. From the data presented, it is apparent that in the presence of local and generalized edema the amount of synovial fluid contained in a joint may be increased. The more marked the edema, whatever its cause, the greater is the synovial fluid content. This finding suggests that such increased formation of synovial fluid occurs where there exists a quantitative imbalance of the normal factors controlling the formation and absorption of interstitial fluid,[5] namely, the colloidal osmotic pressure of the plasma, capillary and tissue pressures and capillary permeability. Synovial fluid specimens from edematous patients were paler and contained fewer cells than the normal. The total solids, viscosity, protein and mucin content of such fluids were lower than normal,[9] signifying that the increased amount of synovial fluid was due to an increased

transudation of plasma water, low in protein content. These results are consistent with our previous contention[2,8] that synovial fluid is a dialysate of plasma water containing mucin, which is added as the plasma water diffuses through the synovial tissues into the joint from the underlying blood-vessels. The evidence to date [2,8] is in keeping with the concept that mucin is formed by the functionally specialized mesenchymal tissues surrounding the joint cavity. Finding a lowered synovial fluid mucin content as a result of increased transudation into the joint implies that the rate of formation of mucin under such conditions is not so accelerated as the rate of transudation of water.

In other instances, the increased synovial fluid contents were the result of inflammatory exudation. In still others, both transudation and exudation seemed to participate, and it was impossible to ascribe the exact rôle.

From the results of the present study, it is apparent that the synovial fluid cytology is frequently altered in patients dying with infection. The synovial fluid cytologic abnormalities found in such cases are increased total nucleated cell and absolute neutrophil counts. Again it was found that elevation of the white cell count in the blood-stream is not, as such, reflected in the synovial fluid. The synovial fluid of patients dying with mild infections, of the type herein described, may show an increase in the absolute neutrophil count as the only evidence of existing synovial tissue inflammation. The fluid of an occasional patient with absent or only minimal clinical signs of infection evinced cytologic changes of inflammation in greater degree than would have been expected from the clinical observations. Since terminal septicemias occur frequently in moribund patients, it is not surprising to find such exceptions.[4,6,7,11]

The interpretation of the significance of the synovial fluid abnormalities was facilitated greatly by examination of tissue specimens from the same joints. Occasionally the synovial tissues showed inflammatory changes although the corresponding synovial fluid cytology was normal. In such instances, the inflammatory focus was situated deep in the subsynovial tissues, suggesting that synovial fluid cytologic alterations resulting from infection are dependent not only upon the severity of the lesion but also on its proximity to the articular cavity. Absence of inflammatory changes in the synovial tissue specimens from joints exhibiting marked cytologic changes probably represents failure on our part to obtain an adequate sample of synovial tissue. As can be seen from Charts 4 and 5, an increase in the absolute neutrophil count is apparently a more sensitive index of inflammation of the articular tissues than is an increase in the total nucleated cell count. A total cell count of more than 400 probably indicates the presence of recognizable inflammation of the articular tissues. Such information should be of aid in the interpretation of pathologic joint effusions and allow

one to predict the extent and severity of the synovial tissue inflammatory lesion.

The severest synovial tissue inflammatory reactions and the most marked cytologic abnormalities were observed in patients dying with proved septicemias. In such cases, microscopic examination of the synovial tissues revealed occasional miliary abscesses containing organisms or inflammatory changes in and about thrombosed blood-vessels. Such inflammatory reactions serve as evidence that synovial membrane inflammation is the result of blood-borne infections.

In most cases the inflammatory changes in the subsynovial tissues were minimal and some distance from the joint cavity. Many of these lesions would probably have resolved completely in a relatively short period of time had the patients lived. This type of synovial tissue reaction would explain the transient aches and pains that occur during the course of acute infectious diseases with associated temporary bacteremia. Indeed, it is surprising that joint infections are not encountered more frequently under such circumstances, a fact which suggests that the tissues surrounding the joint posssess high resistance to infection. The occurrence and degree of joint infection appear to depend more upon the adequacy of the normal defense mechanisms in the synovial tissues and the severity of the infection than the type of infective organism. The most marked joint reactions observed in this study would probably have been recognized as specific infectious or septic arthritis had the patients not been moribund.

Conclusions. From a study of 156 postmortem synovial fluids and 49 synovial tissue specimens obtained from individuals dying with varying degrees of infection and edema, the following conclusions can be drawn:

1. The amount of synovial fluid in the knee joints of individuals with peripheral edema is usually increased.

2. The synovial fluid obtained from the joints of edematous patients contains fewer nucleated cells than does normal fluid and its total solids, viscosity, protein and mucin contents are reduced.

3. The total nucleated cell counts and the absolute number of neutrophils in synovial fluid specimens obtained from patients dying with varying degrees of infection are frequently increased. The most marked cytologic abnormalities are observed in patients dying with severe infections and associated septicemias.

4. An increase in the absolute number of neutrophils is a more reliable indication of the existence of synovial tissue inflammation than are increased total nucleated cell counts or random biopsies.

5. The synovial fluid abnormalities resulting from synovial tissue infections vary with the severity of the synovial lesion and its proximity to the joint space.

6. The results of the present study suggest that the joint aches

and pains occurring in various infectious diseases may be the result of synovial tissue inflammatory changes caused by blood-borne infections. The majority of such inflammatory lesions are presumably mild in nature and therefore resolve readily. The marked synovial tissue reactions observed in patients dying with septicemia are of the type observed in early cases of specific infectious or septic arthritis.

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STUDIES OF THE B VITAMINS IN THE HUMAN SUBJECT.*

I. The Intake of Thiamine and its Relation to Other Dietary Constituents in Food Selected by the Normal Subject.

By K. O'Shea Elsom, M.D.,

ASSOCIATE IN MEDICINE, UNIVERSITY OF PENNSYLVANIA MEDICAL SCHOOL; RESEARCH FELLOW, PHILADELPHIA GENERAL HOSPITAL,

AND

Thomas E. Machella, M.D.,

INSTRUCTOR IN MEDICINE AND IN PHYSIOLOGY, UNIVERSITY OF PENNSYLVANIA MEDICAL SCHOOL; RESEARCH FELLOW, PHILADELPHIA GENERAL HOSPITAL, PHILADELPHIA, PA.

The purpose of this study was to determine the actual thiamine intake of a group of normal persons who were permitted to select from an adequate available diet the amount and type of food which they desired. Such information, it was thought, would indicate to what extent the food chosen under such circumstances contains the amount of the vitamin regarded as necessary to meet bodily needs, to what extent individual preference determines the amount ingested and to what extent the amount normally selected by the individual varies from day to day.

* Under this title will appear serially in this Journal several articles from the Thompson Vitamin Clinic of the Philadelphia General Hospital. This Clinic, sponsored by the Gastro-Intestinal Section (Kinsey-Thomas Foundation) of the Medical Clinic, University of Pennsylvania Hospital, and by the Philadelphia General Hospital, is generously supported by Mrs. Arthur W. Thompson. The work is being supervised by an advisory committee, consisting of Drs. Truman G. Schnabel, William G. Turnbull, J. H. Clark, Walter G. Karr, George Wilson and T. Grier Miller, and the chemical studies are being carried out under the supervision of Dr. John G. Reinhold, Chief of the Division of Biochemistry of the Philadelphia General Hospital. Miss L. M. Johnson and Miss Elizabeth Miller, respectively Directress of Nurses and Dietitian of the Philadelphia General Hospital, have coöperated. The vitamin preparations, except when otherwise noted, have been supplied by Merck & Co.

It was found in a selected group that the thiamine intake exceeded the theoretical requirement in most instances, but varied greatly among individuals and in the same persons from day to day; that the consumption of protein and of thiamine were directly related, and that some correlation existed between the intake of fat and of thiamine, whereas no correlation was demonstrable between the thiamine, on the one hand, and the total caloric or carbohydrate intake, on the other.

Methods. Seven women, essentially normal and resident in the Vitamin Ward of the Philadelphia General Hospital, were permitted to select from an adequate diet the type and quantity of food which they desired for periods of from 15 to 39 days. All of the food was carefully weighed. The total daily intake of thiamine was calculated from the Cowgill tables,[5] and also from figures compiled by Williams and Spies.[26] Corroboration of the calculated values was obtained by chemical analysis of daily diets on 3 occasions, using a modification of the thiochrome method of Hennessy and Cerecedo[15] and Hennessy[14] as described elsewhere.[9] The protein, fat, carbohydrate and caloric values were obtained from standard tables.[3] The theoretical thiamine requirement for each individual was calculated in micrograms of thiamine according to the Cowgill formula,[5] $\frac{\text{Vitamin } B_1}{\text{Calories}} = 0.0000284 \text{ W gm.} \times 0.166$, using the average caloric intake and average body weight during the time of observation. The subjects were weighed daily under standard conditions and were observed closely for the development of symptoms and abnormal physical signs. The fluid intake was maintained at 1500 cc., and 24-hour collections of urine were obtained. Analyses for the thiamine in the urine were made by the same method as for the food and will be reported in another communication.[9]

TABLE 1.—THIAMINE REQUIREMENTS AND INTAKE ON SELF-SELECTED DIET.

Subject.	Length of observation (days.)	Age (years).	Average body weight* (kg.).	Theoretical requirement for thiamine (μg.).	Av. daily intake of thiamine (μg.).
1	30	64	57.5	524	663
2	30	65	59.0	503	691
3	30	38	78.0	715	760
4	30	52	65.0	610	784
5	30	52	43.0	380	817
6	15	36	71.7	646	842
7	39	45	54.3	574	973
Average	25	50	60.0	565	789

* Subjects 3 and 6 lost 1.3 and 0.8 kg. respectively; the others either maintained or gained weight. The group as a whole gained 0.5 kg.

Results. 1. *Thiamine Intake in Relation to Theoretical Requirement.* The average daily intake of thiamine by the entire group was 789 μg., while the average theoretical requirement was 565 μg. (Table 1). The intake thus exceeded the requirement by 40% in the group as a whole. The individual variation in this respect, however, was great. For example, Subject 3 exceeded her need for the vitamin by only 6% (Chart 1), and for approximately one-half the period of observation failed to consume the required

amount. Likewise, Subject 1, whose average intake was 26% above her requirement, failed to obtain an adequate amount for one-quarter of the period. At the other extreme, Subject 5 exceeded her theoretical requirement by 115% and never failed to consume less than 40% above that amount. Thus it is apparent that individual food habits are an important determining factor in the margin of safety maintained with respect to thiamine intake.

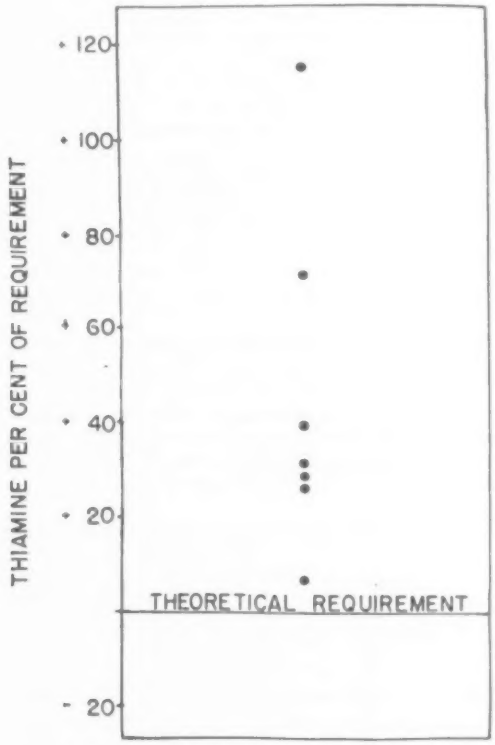

CHART 1.—Average daily intake of thiamine as per cent of requirement for each member of the group.

The subjects with a minimal intake showed clinical evidence of a thiamine deficiency during the time that the food selected by them was below their requirement. Thus Subject 1, who was known to have taken a borderline diet prior to admission to the hospital, complained of easy fatigability, heartburn, epigastric distress and pains in the extremities and showed tenderness to pressure over her peripheral nerves and edema. These symptoms and signs regressed when the intake of thiamine was adequate. Subject 3, whose inadequate selection of foods was of shorter duration, developed the phenomena of deficiency later than Sub-

ject 1. These observations, together with the fact that at no time did the other 5 subjects show any evidence of deficiency tend to confirm the accuracy of the calculations of theoretical requirement.

TABLE 2.—FOOD SELECTED BY SUBJECTS 1 AND 5, OCTOBER 6, 1940.

Article.	Subject 1.						Subject 5.					
	Wt. (gm.).	Prot. (gm.).	Fat (gm.).	Carb. (gm.).	Cal.	Thiamine (μg.).	Wt. (gm.).	Prot. (gm.).	Fat (gm.).	Carb. (gm.).	Cal.	Thiamine (μg.).
Breakfast:												
Egg	50	7	5	..	75	48	50	7	5	..	75	48
Cream of Wheat	166	3	..	24	116	16						
Bread	11	1	..	6	29	5	33	3	..	17	86	13
Milk	..	..	..	..	..	..	150	5	6	8	105	81
Lunch:												
Ham (boiled)	99	18	5	..	149	197	99	18	5	..	149	197
Potatoes (boiled)	100	3	..	25	110	67	100	3	..	25	110	67
Peas	60	2	..	6	30	78	60	2	..	6	30	78
Bread	..	..	..	..	..	..	22	2	..	11	57	9
Butter	12	..	10	..	96	16	12	..	10	..	96	16
Custard	75	3	4	9	83	48	75	3	4	9	83	48
Milk	..	..	..	..	..	..	150	5	6	8	105	81
Supper:												
Pea soup	150	7	2	15	135	81	75	4	1	8	68	41
Frankfurter	..	..	..	..	..	..	120	23	22	1	312	334
Bread	22	2	..	11	57	9	44	4	1	23	114	18
Butter	6	..	5	..	48	8	12	..	10	..	96	16
Milk	..	..	..	..	..	..	180	6	7	9	126	97
Sugar (total)	74	..	..	74	289	..	2	..	..	2	8	
Total	..	46	31	170	1217	573	..	85	77	127	1620	1144

No correlation between the individual theoretical requirement for thiamine and its intake was observed. For example, Subject 5 had the lowest requirement, but her average intake was greater than that of any but two of the group (Table 1). This is further strikingly demonstrated by the daily records of thiamine intake by Subjects 1 and 5 (Chart 2). It will be noted that the individual with the lower requirement consistently selected food of greater thiamine content. For example, on the first day of observation Subject 1, whose requirement was 524 μg., obtained 573 μg. from the available diet, while Subject 5, whose requirement was 380 μg., obtained 1144 μg. Analysis of the food intake on that day (Table 2) shows that, whereas the higher intake of thiamine was associated with a greater total number of calories, the difference could not be explained entirely by this fact, but was the result of a greater preference on the part of this subject for foods rich in thiamine. Thus it would appear that food habits rather than needs determined the level of thiamine intake in this group.

2. *Daily Variation in Thiamine Intake.* In addition to the wide divergence in intake between the several members of the group, a large variation occurred from day to day in each individual. Two factors appeared to be responsible for this. Firstly, differences in

the thiamine content of the available food produced fluctuations in intake. For example, on a given day when pork, which is rich in the vitamin, was included in the diet, a sharp rise in the thiamine intake was observable for each member of the group. Secondly, individual food habits modified the intake, depending upon the individual's like or dislike for certain articles of the diet. The magnitude of this individual variation was greatest in Subject 5, whose intake on one day exceeded the mean by 72% and on another fell below it by 60%. At the other extreme was Subject 1 who never departed from the mean intake by more than 50%. It is scarcely surprising therefore to find in the literature[2,4,18,21,24] evidence of wide fluctuations in thiamine output among normal persons. Such figures are without great significance unless they are considered in relation to the thiamine intake which our observations prove fluctuate widely.

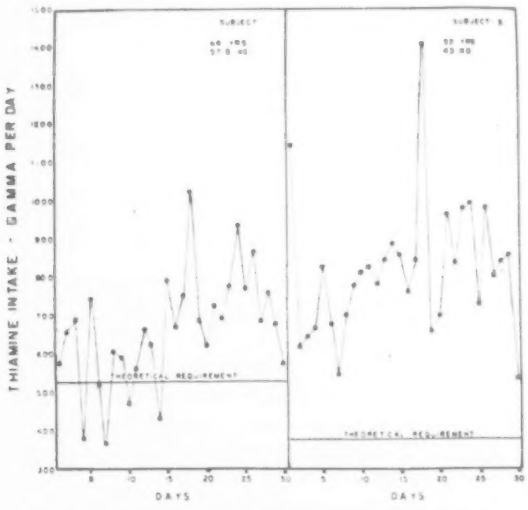

CHART 2.—Daily intake of thiamine in relation to requirement for Subjects 1 and 5.

3. *The Relation of Thiamine Intake to That of Other Dietary Constituents.* A linear relationship existed between the daily intake of protein and of thiamine (Chart 3). This relationship, found to be statistically valid, existed for the group as a whole as well as for each individual member.* This was not, as might at first appear,

* We are indebted to Dr. E. D. Burdick for making the statistical calculations to prove the validity of this relationship. The coefficient of correlation was 0.728 calculated by the formula:

$$\frac{N\Sigma XY - (\Sigma X)(\Sigma Y)}{\sqrt{[N\Sigma X^2 - (\Sigma X)^2][N\Sigma Y^2 - (\Sigma Y)^2]}}$$

The variance around the regression line was 19.11 as calculated by the formula:

$$\sqrt{\frac{\Sigma Y^2}{N} - \left(\frac{\Sigma Y}{N}\right)^2}$$

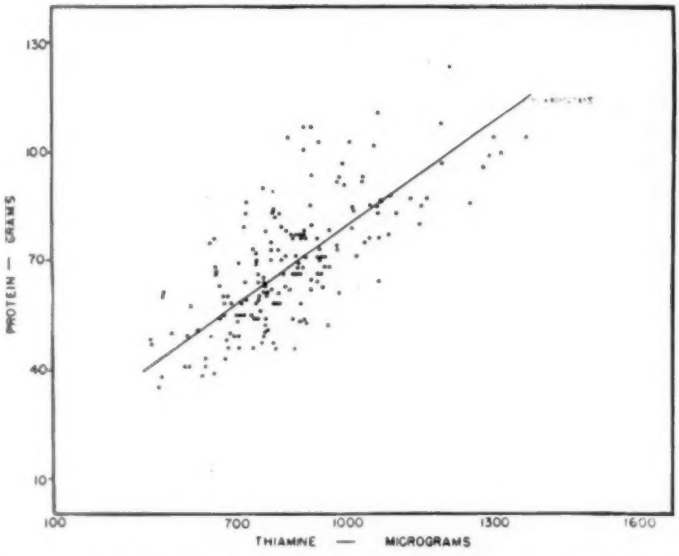

CHART 3.—Protein in relation to thiamine in food selected by the group. Each point in the diagram represents the total protein and thiamine consumed by each individual during each day.

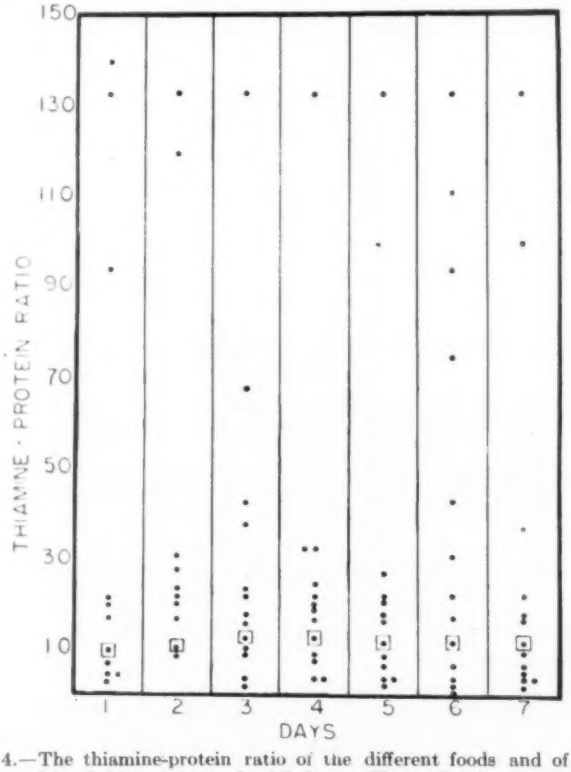

CHART 4.—The thiamine-protein ratio of the different foods and of the total food consumed by Subject 6 for each of 7 days. The enclosed points indicate the thiamine-protein ratio of the total food consumed each day.

simply an expression of the fact that protein foods by and large are rich in thiamine. Analysis of foods making up the diet shows that some foods, such as noodles, rice and cheese, provide a large amount of protein but a small amount of thiamine. Conversely, milk, apricots, pork, potatoes, oatmeal, raw cabbage, liver and Ralston cereal fortified with wheat germ, appearing often in the diet, are proportionally richer in thiamine than in protein. Thus great variability in the thiamine-protein ratio existed for individual foods, while it was constant for the total food consumption on each day (Chart 4). Hence a linear relationship between thiamine and protein need not necessarily have existed. When, however, the subject was given a free choice of protein foods, a mixture was

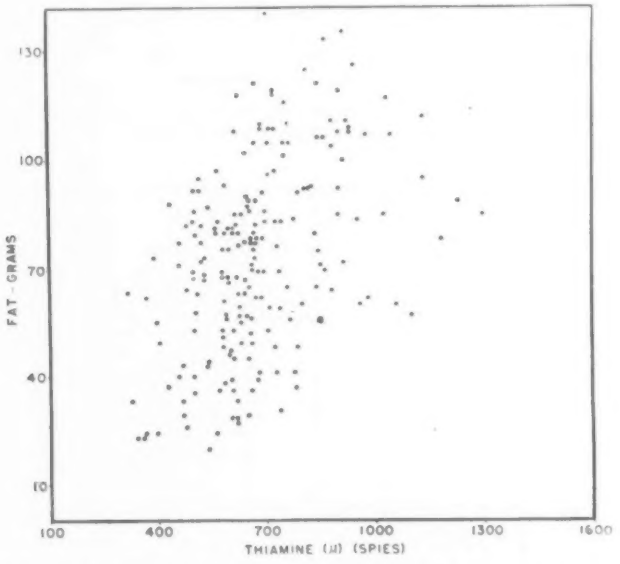

CHART 5.—Fat in relation to thiamine in food selected by the group. The values for thiamine used in preparation of this figure were those given by Williams and Spies.[26] No thiamine value for fat is included in these values and therefore the positive correlation between fat and thiamine here demonstrated is independent of the relationship inherent in the Cowgill figures where thiamine values are assigned to fat on the basis of the known thiamine-sparing action of fat.

selected which provided a fairly constant ratio of protein to thiamine and resulted in the linear relationship described.* Whether this relationship obtains in a deficiency-producing diet is a subject that deserves separate study.

* It is probable, also, that a similar relationship existed between protein and other members of the B complex in this diet. However, figures for the concentration of these substances in foods are not sufficiently complete to permit calculation of the dietary content.

A positive correlation, though less definite than in the case of protein, was observed between the consumption of fat and of thiamine (Chart 5). No such correlation, however, was observed

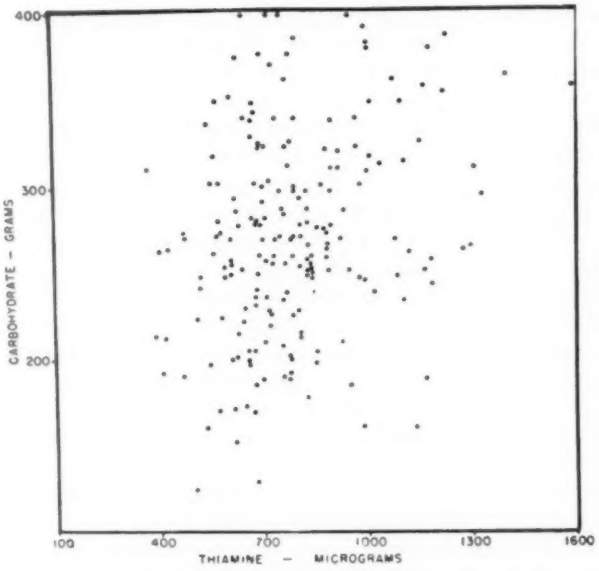

CHART 6.—Carbohydrate in relation to thiamine in food selected by the group.

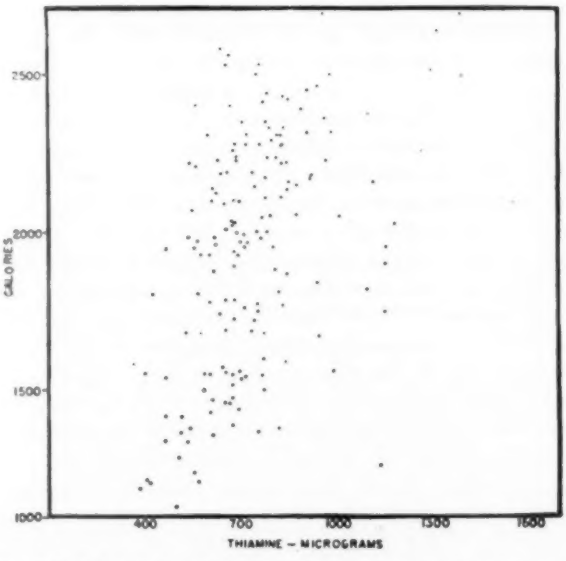

CHART 7.—Caloric intake in relation to thiamine in food selected by the group.

between daily carbohydrate and thiamine (Chart 6) or between total daily caloric and thiamine intake (Chart 7).

Discussion. The widely different amount of thiamine obtained from the same available food by these subjects demonstrates clearly that the adequacy of the vitamin intake cannot be determined alone from a knowledge of its content in the available food. That a vitamin deficiency may develop even in the presence of an adequate food supply and that this deficiency may be determined primarily by individual food habits are demonstrated by the failure, on frequent occasions, of two of the subjects to meet their needs for thiamine while other members of the group, living under identical conditions, obtained amounts which greatly exceeded their requirement. This observation may explain also the seemingly puzzling fact that deficiency disease often afflicts only one or two members of a household in which the remainder are healthy.

The food consumed by these subjects was inexpensive, costing approximately $2.80 per week per person as purchased from the usual retail sources. That it supplied thiamine in more than an adequate amount for any normal person is demonstrated by the fact that one subject obtained from it an average of 973 μg. which is an amount sufficient to meet the theoretical requirement of an individual weighing 181 pounds.[5] Stiebeling and Phipard[23] have recently reported that a weekly expenditure of $3.50 to $3.12 for food in the North Atlantic States resulted in poor diets in 53% of the cases while others[16,22] have likewise suggested that the diet of the average American in the low income group is borderline with respect to thiamine. That such observations based only on several surveys of available food, are at variance with our objective idea on individual subjects tends further to emphasize the fact that, except in grossly limited diets, the adequacy or inadequacy of a given diet can be determined only by a careful analysis of food intake by the individual in relation to his requirement.

Whether the quantitative relationship between protein and thiamine which existed in the food consumed by these normal subjects is an essential of a normal diet requires further study. That such a quantitative relationship between dietary factors may be of importance has recently been demonstrated in the case of cystine and choline.[11a,b,c,d] There is evidence also that the ability to utilize protein is dependent on the presence in the diet of a proportionate amount of the vitamin B complex. Thus in 1924 Hartwell[12a] and later Reader and Drummond[20] and others[6,12b,13] found that when the protein was increased out of proportion to the B complex in the diets of young rats growth was curtailed. The component of the vitamin B complex concerned in this response has not been identified.[10,19] In humans, observations bearing on the relationship of the B vitamins to nitrogen metabolism are few. Elsom, Dickey and Chornock,[8] studying the absorption of nitrogen

in subjects with ulcerative colitis, could find no correlation between clinical evidence of vitamin deficiency and decreased nitrogen absorption. On the other hand, one of us,[7a] studying a human subject maintained under constant conditions of experimental B complex deficiency, observed a positive nitrogen balance coincident with gain in body weight following addition of yeast to the diet. On another occasion[7b] urinary nitrogen excretion was found decreased during B complex deficiency.

The quantitative relationship between fat and thiamine in these diets recalls the work of Whipple and Church[25] and more recently of McHenry and Gavin[17] and others[1] who have suggested that thiamine is concerned in the metabolism of fat.

Summary. 1. Seven subjects, maintained under controlled conditions, were given access to an adequate diet from which they selected the type and quantity of food desired.

2. The average daily intake of thiamine exceeded the theoretical requirement for that vitamin by 40% for the group as a whole, but the individual intake ranged from 6 to 115% above the requirement. In addition to a wide divergence of thiamine intake among the several members of the group, a large variation occurred from day to day in the same individual.

3. Individual food habits appeared to be an important determining factor in the margin of safety maintained with respect to the thiamine in the diet.

4. A linear relationship existed between the daily intake of protein and of thiamine, both for the group as a whole and for each individual. This relationship resulted from the selection of foods of both high and low protein content in such a proportion as to produce a fairly constant ratio, but one that did not pertain to the individual foods consumed.

5. A positive correlation, of lesser degree than that between the protein and the thiamine, was observed between the fat and the thiamine content of the diet. No correlation existed between the carbohydrate and the thiamine or between the calorie intake and the thiamine of the diet.

We wish to express our appreciation of the faithful nursing care rendered by Misses Ruth Conrad, Ruth Kruger, Elizabeth Villella and Ruth Jett; of the assistance with the diets given by Miss Alice Knudsen, and of the secretarial assistance provided by Miss Ellen Jones.

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STUDIES OF THE B VITAMINS IN THE HUMAN SUBJECT.

II. URINARY EXCRETION OF INGESTED THIAMINE IN PATIENTS WITH CHRONIC HEPATIC DISEASE.*

BY THOMAS E. MACHELLA, M.D.,

INSTRUCTOR IN MEDICINE AND IN PHYSIOLOGY, UNIVERSITY OF PENNSYLVANIA MEDICAL SCHOOL; RESEARCH FELLOW, PHILADELPHIA GENERAL HOSPITAL,

AND

K. O'SHEA ELSOM, M.D.,

ASSOCIATE IN MEDICINE, UNIVERSITY OF PENNSYLVANIA MEDICAL SCHOOL; RESEARCH FELLOW, PHILADELPHIA GENERAL HOSPITAL.

With the technical assistance of CHARLOTTE S. CHORNOCK, PH.D.,

PHILADELPHIA, PA.

(From the Thompson Vitamin Clinic of the Philadelphia General Hospital and of the Kinsey-Thomas Foundation of the University of Pennsylvania Medical School.)

THE influence of hepatic disease on the excretion of thiamine in the urine has not been definitely ascertained. The concept of its phosphorylation in the liver, as based on the studies of Ochoa and Peters[7] and of Westenbrink and Goudsmit,[11a] has led to the opinion that there should occur a greater urinary excretion of the vitamin in liver disease, and this idea has been supported by Borson,[1] who noted an unusually high excretion in 3 patients with hepatic cirrhosis following the administration of daily test doses. On the contrary, Robinson, Melnick and Field[8] found low values for its urinary excretion in 3 patients with hepatic disease following the administration of a single test dose. They attributed this to a previous suboptimal intake in their patients. In an attempt to obtain more data on the problem, we have made simultaneous observations on subjects with and without hepatic disease while on a basal diet and while on such a diet supplemented with small additional amounts of thiamine chloride orally administered.

* Presented before the American Federation of Societies for Clinical Research, May 5, 1941, at Atlantic City, N. J.

Procedure. Three of the 6 female subjects in the vitamin ward of the Philadelphia General Hospital, who were used for this study, had clinical and laboratory* evidence of chronic hepatic disease; the remaining 3 had no such disease and were used as controls. All had satisfactory renal function as judged by the standard urea clearance test, and none had any signs of a B-complex deficiency.

The thiamine requirement for each subject was calculated from Cowgill's[2] formula, and this amount together with an adequate number of calories and the other essentials were included in a basal diet. After a preliminary observation period of about 2 weeks, during which they were allowed access to as much of the house diet as they wished, the subjects were placed on the basal diet for 16 to 37 days, and later it was supplemented with varying amounts of additional thiamine, to as much as 1000 μg. daily. The total daily fluid intake was limited to 1500 cc., and 24-hour urine specimens were collected. The urine, as voided, was placed in individual bottles kept in a refrigerator, glacial acetic acid being added to prevent decomposition of the thiamine. The amount of thiamine in each 24-hour specimen was determined by a modification of the thiochrome method of Hennessy and Cerecedo[5] to be described in another paper.[3]

Results. 1. *Excretion on the Basal Diet.* No significant difference was observed in the percentage of the thiamine excreted in the urine by the patients with hepatic disease and by the controls while on the basal diet, all 6 excreting 6.5 to 8.7% of the amount ingested (Table 1).

TABLE 1.—URINARY EXCRETION OF THIAMINE.

Case.	On basal diet.		On basal diet plus added thiamine.	
	Average daily thiamine intake (μg.).	Average daily urinary excretion (%).	Average daily thiamine intake (μg.).	Average daily urinary excretion (%).
1. Laennec's cirrhosis (F.R.)	715	8.7 (4.0–13.5)	1752	14.3 (3.3–27.2)
Control (A.T.)	527	6.5 (4.0–7.8)	1532	17.4 (7.5–26.0)
2. Banti's syndrome (A.H.)	652	8.2 (6.1–14.2)	1641	13.8 (10.0–16.5)
Control (M.H.)	422	8.2 (4.7–10.1)	1459	20.4 (14.8–25.2)
3. Banti's syndrome (Post-splenectomy) (S.L.)	651	7.2 (4.0–10.1)	1651	20.7 (18.8–22.0)
Control (J.Z.)	706	8.3 (4.3–11.9)	1655	26.5 (23.2–30.0)

Average total daily thiamine intake and percentage of this excreted in the urine while on basal diet and when such a diet was supplemented with 1000 μg. of thiamine daily by mouth. Figures in parenthesis represent range of excretion.

2. *Excretion on the Basal Diet Supplemented with Thiamine. A.* A patient with Laennec's cirrhosis and her control were given by mouth 1000 μg. of thiamine chloride† daily in addition to the basal diet for a period of 14 days. The excretion of the patient with the liver damage rose less rapidly and to a lower level than did

* An elevated icteric index, retention of bromsulphalein and impairment of hippuric acid synthesis.

† Generously supplied by Merck & Co., Rahway, N. J.

that of the control. The average percentage excretion for the 14-day period was 17.4% for the control and 14.3% for the cirrhotic.

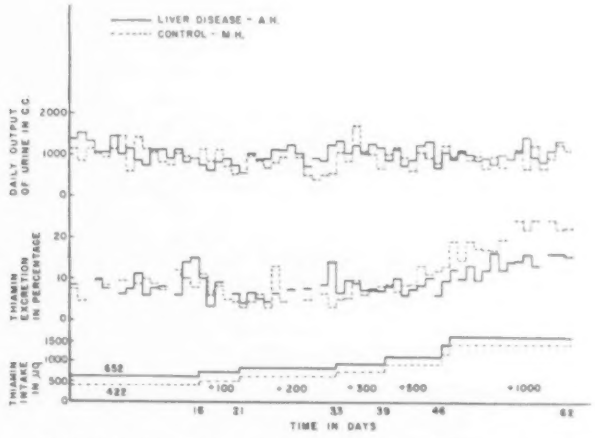

CHART 1.—Daily thiamine intake, urinary excretion and daily 24-hour urine volume output of a patient with Banti's syndrome and of her control.

B. To a patient with Banti's syndrome and to her control the supplements of thiamine were added in increments gradually increasing until 1000 μg. were being administered daily. In this instance also the excretion of the patient with the damaged liver lagged behind and did not reach the height of the control. When 1000 μg.

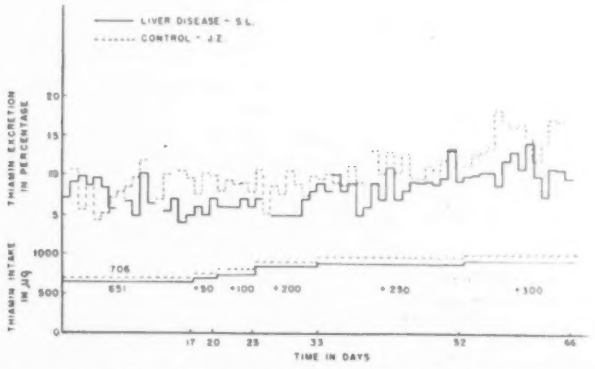

CHART 2.—Daily thiamine intake and urinary excretion of a patient with a post-splenectomy Banti's disease and of her control.

daily were being added, the patient with hepatic disease excreted an average of 13.8% of the ingested thiamine as compared to 20.4% for the control. Furthermore, the total volume of the urine excreted daily over the entire period bore no relationship to the percentage of the thiamine excreted (Chart 1).

C. To a patient with postsplenectomy Banti's syndrome and to her control the supplements were given in smaller increments in order to determine whether or not a slow increase in the thiamine intake would dispel the difference in excretion observed in the previous experiments. Though no lag in the excretion for the subject with liver disease occurred, the level of excretion was persistently lower than in the control. When each subject received a daily supplement of 1000 μg., the patient with the damaged liver excreted on the average 20.7% of the ingested thiamine while the control excreted 26.5% (Chart 2, Table 1).

Comment. These data indicate that when supplements of thiamine, up to 1000 μg. daily, were added to the basal diet the patients with hepatic disease excreted less than did the controls. Such observations are not in accord with the claim[1] that, because phosphorylation of thiamine occurs in the liver, the urinary excretion of ingested thiamine should be higher in the subjects whose livers are damaged. It must be admitted, however, that, providing its absorption in the intestinal tract is not impaired, the amount of thiamine phosphorylated may be determined by the type and extent of liver damage.

The following possibilities in explanation of the lessened excretion in our hepatic cases are suggested:

1. *Disturbed Renal Function.* That this was not the important factor in our subjects is indicated by the satisfactory urea clearance in each of them. Furthermore, daily determinations of the volume and the specific gravity of the urine showed no significant deviations.

2. *Previous State of Tissue Unsaturation.* The patients with liver disease received a B-complex preparation prior to their becoming the subjects for our study. Furthermore, while in the vitamin ward, they ingested from 600 to 1500 μg. of thiamine contained in food per day for at least a month before receiving the supplements of thiamine and during that time the urinary excretion of thiamine was within normal range. Thus a previous state of unsaturation cannot be claimed as being responsible for the decreased level of excretion in the subjects with hepatic disease.

3. *Absorption from the Intestinal Tract.* An impairment of absorption from the intestinal tract must be seriously considered, since all 3 of the liver cases had clinical evidence of portal back pressure. Such a factor is known to impair the absorption of substances which are transferred across the intestinal wall by diffusion, and it has recently been shown by Stockholm *et al.*[10] that, in rats, thiamine is so absorbed. Furthermore, using very high test doses of thiamine, Westenbrink and Goudsmit[11b] and also Borson[1] observed that, following oral administration, unlike parenteral administration, the percentage of thiamine excreted in the urine varies inversely with the size of the dose administered. This unabsorbed thiamine appears in the feces, as has been demonstrated by Leong,[6] Shultz *et al.*[9] as well as in our clinic.[4] Further studies using an intestinal intubation

technique which we have planned, are necessary to settle this matter finally.

Summary. On a basal diet no essential difference occurred in the percentage of ingested thiamine excreted in the urine of patients with hepatic disease and in that of control subjects. When such a basal diet was supplemented with thiamine, not over 1000 μg., the patients with liver disease excreted less. The possibility is discussed that an impairment of intestinal absorption in our patients with hepatic disease, consequent upon portal back pressure, accounts for this difference.

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PROTEIN-BOUND IODINE IN BLOOD.

V. NATURALLY OCCURRING IODINE FRACTIONS AND THEIR CHEMICAL BEHAVIOR.*

BY A. MERTON BASSETT, M.D.,

RESEARCH FELLOW, HARVARD MEDICAL SCHOOL; ASSISTANT RESIDENT PHYSICIAN, THORNDIKE MEMORIAL LABORATORY; ASSISTANT IN MEDICINE, BOSTON CITY HOSPITAL

ALBERT H. COONS, M.D.,

RESEARCH FELLOW, HARVARD MEDICAL SCHOOL; ASSISTANT RESIDENT PHYSICIAN, THORNDIKE MEMORIAL LABORATORY; ASSISTANT IN MEDICINE, BOSTON CITY HOSPITAL,

AND

WILLIAM T. SALTER, M.D.,

ASSISTANT PROFESSOR OF MEDICINE, HARVARD MEDICAL SCHOOL; ASSOCIATE PHYSICIAN THORNDIKE MEMORIAL LABORATORY; JUNIOR VISITING PHYSICIAN, BOSTON CITY HOSPITAL,

With the technical assistance of SOPHIA M. SIMMONS, S.B.,

BOSTON, MASS.

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.)

DURING the past two decades microchemical methods for the estimation of the small amounts of iodine that are found in the

* This investigation was aided by grants to William T. Salter from The International Cancer Research Foundation and from the Milton Fund of Harvard University.

circulation have improved steadily, so that the values obtained by rather different procedures now corroborate one another. Although the absolute magnitude of these figures is still open to question, the reliability of the methods now available justifies a qualitative study of different iodine fractions in the blood. In previous studies from this laboratory the thyroidal activity of iodoproteins has been described.[10a,b 12,16] It is the purpose of this communication to describe certain protein-bound iodine fractions which may be separated from human and animal blood.

In 1900 Gley and Bourcet[6] studied the iodine present in amounts of dog plasma as large as 1 liter. They found an average value of 5 μg. per 100 cc. and upon dialysis they were able to remove only a negligible portion of the total iodine. They concluded, therefore, that the naturally occurring iodine in the plasma is bound to protein. Since then, numerous other approaches to this problem have been made, of which it is possible to cite here only a few examples. A more detailed review has been published elsewhere.[15] Bier and Roman[2] dialyzed ox serum for 10 days and were able by this procedure to remove only about one-fourth of the iodine present. Similarly, Leipert[8] found that ultrafiltrates of horse serum yielded less than half of the iodine in the filtrate. More recently, Trevorrow[20] found that the major portion of the plasma iodine was precipitated with the plasma proteins either by heat coagulation with acetic acid[1] or by precipitation with zinc sulphate and sodium hydroxide.[19] Although a great deal of work reported in the literature has to do with the use of organic solvents, *e. g.*, alcohol or acetone, to remove protein-bound iodine, it should be emphasized that this method has not been employed in the present observations because organic solvents yield variable results according to the details of the procedure followed. This problem has been discussed in detail elsewhere.[15]

General Methods. *Collection of Blood.* When fresh blood was drawn, extreme care was exercised to prevent contamination with iodine through the use of chemically clean, iodine-free glassware and blood anticoagulant (potassium oxalate or heparin).

Determination of Iodine. Two methods were used to determine total iodine in the different fractions. These were (*a*) the æration procedure of Fashena and Trevorrow[5] following oxidation with dichromate; and (*b*) the distillation method of Riggs and Man[14] following oxidation with permanganate. The two series of values coincided so closely that no distinct difference could be discerned.

The data presented in this communication concern mainly the plasma; whole blood was avoided because the distribution ratio of "hormonal" iodine between the intra- and extra-corpuscular protein components of whole blood is still disputed. Preliminary experiments with heat coagulation yielded results which are summarized in Table. 1 The whole blood was laked and the protein components separated as follows: The plasma proteins were precipitated by heat at pH 5.3 and the hemoglobin by heat at pH 6.6; the iodine bound to each protein fraction was then determined. The separated plasma was analyzed as described later. From the hematocrit

values given in Table 1, the protein-bound iodine content of the erythrocytes may be calculated if desired. The problem of the natural iodine content of red cells, however, is still under discussion.[7,13] Nevertheless the results in the table demonstrate that over a considerable range of physiological thyroid activity the use of plasma gives a wider spread in iodine values and thus affords a greater sensitivity of the chemical method. These data are not presented, therefore, as the final solution to the question of intracorpuscular iodine. Rather, they constitute a valid reason for avoiding the use of whole blood when the present technique is employed.

TABLE 1.—PROTEIN-BOUND "P" IODINE IN WHOLE BLOOD AND PLASMA.

Case No.	Sex.	Age.	B.M.R.	Hematocrit.	Blood iodine. Whole blood μg. per 100 cc.	Blood iodine. Plasma, μg. per 100 cc.	Ratio plasma/whole blood.
			Myxedema.				
4	M	42	−32	41.6	3	3	0.9
3	F	41	−37	35.4	3	2	0.6
1	F	44	−45	33.0	2	2	0.9
7	F	73	−42	..	..	2	
6	F	55	−18	..	..	1	
11	F	62	−21	..	..	2	
	Average		−33	..	3	2	0.8
			Euthyroidism.				
109	M	19	−9	40.1	5	6	1.1
82	F	31	+21?	44.9	6	5	0.9
99	F	41	+36	49.8	6	6	1.0
101	F	25	0	40.4	6	5	0.9
107	M	52	−3	48.6	5	5	1.0
	Average		+2	..	6	5	1.0
			Hyperthyroidism				
41	F	59	+36	43.6	9	11	1.3
39	M	19	+45	42.5	9	12	1.3
34	F	31	+50	42.8	9	11	1.3
A.M.*	F	47	+61	39.0	8	13	1.7
	Average		+48	..	9	12	1.4

* B.C.H. No. 981559.

IODINE DISTRIBUTION IN PLASMA: *Separation of Inorganic ("I") from Protein-bound ("P") Iodine.* Plasma was separated by centrifugation shortly after the blood was drawn and treated as follows:

One volume of plasma (usually 10 cc.) was added dropwise with constant stirring to a 50 cc. centrifuge tube containing 3 volumes of 0.02 normal acetic acid. The tube was placed in a water bath which was brought to boiling within a period of 10 minutes, and the mixture was stirred constantly throughout the process of coagulation. By this time the supernatant liquor appeared water clear and the precipitate showed definite flocculation. The final pH was 5.3. After cooling, the tube was covered with a few layers of gauze and centrifuged for 15 minutes at 2500 r.p.m. The supernatant fluid was removed by decantation or siphoning and was analyzed for iodine. This fraction was designated as inorganic, or "I" iodine. The protein precipitate was washed once with the same volume of 0.02 normal acetic acid as was used in the precipitation. After a second centrifugation the supernatant liquor was discarded and the protein precipitate was analyzed for iodine; this fraction was designated as the protein-bound or "P" iodine. When it seemed likely that the inorganic fraction was unusually high, as discussed later, the protein coagulum was washed four times in the same manner.

Inorganic Iodine. The values for "inorganic" iodine of human plasma were obtained as described above and are shown in Figure 1. The inorganic iodine, "I," constitutes roughly one-third of the normal total plasma iodine as determined by this method. It varies between 0.5 and 4 μg. per 100 cc.; the mean value is approximately 2 μg. per 100 cc. It is apparent that the value of inorganic iodine is relatively constant despite wide variations of thyroid activity. The inorganic iodine, however, is increased markedly whenever the patient receives unusual amounts of iodine from an external source. Such sources may be diets high in iodine, medication, external applications of iodine to the skin, inhalation of

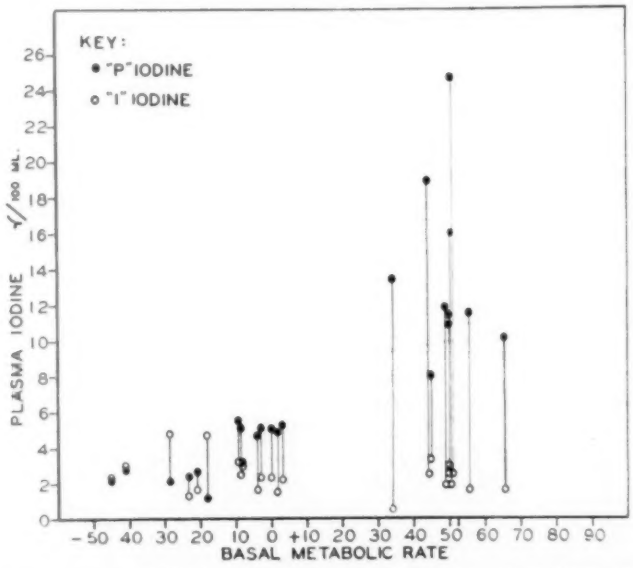

FIG. 1.—In cases ranging from myxedema to marked hyperthyroidism, the inorganic "I" iodine tends to remain constant, although the protein-bound "P" iodine rises as thyroid activity increases.

iodine vapors or diagnostic procedures employing radio-opaque materials rich in iodine. Under such circumstances the inorganic iodine may reach values exceeding 1000 μg. per 100 cc. In one instance tincture of iodine applied to the skin in preparation for lumbar puncture was sufficient to raise the inorganic iodine to nearly 2000 μg. per 100 cc. The elevation of "I" iodine, however, following a "standard dose"* of iodine tends to be less marked in cases of hyperthyroidism than in euthyroid or hypothyroid cases. This point will be discussed later.

PROTEIN-BOUND IODINE. *Distribution of "P" Iodine in Successive Fractions of the Plasma Proteins.* McMeekin[11] has described the

* Five minims of saturated solution of potassium iodide daily for 6 days.

precipitation seriatim of successive protein components of horse serum through the use of ammonium sulphate. In the present studies such fractions, obtained through the courtesy of Dr. McMeekin and also prepared in this laboratory by his procedure, were analyzed for total protein and for protein-bound iodine. The fractions were separated at approximately the following concentrations of ammonium sulphate: 1.6 M.,* 2.1 M., 2.8 M. and 4.0 M. In addition, the albumin fraction soluble at 2.1 M., but insoluble at 2.8 M. was further fractionated into two subfractions, (*a*) that removed at 2.4 M. and (*b*) that removed at 2.8 M. The former of these two was fractionated further by removal of crystalbumin. In this manner a spectrum of the plasma or serum was obtained showing simultaneous distributions of proteins and protein-bound iodine.

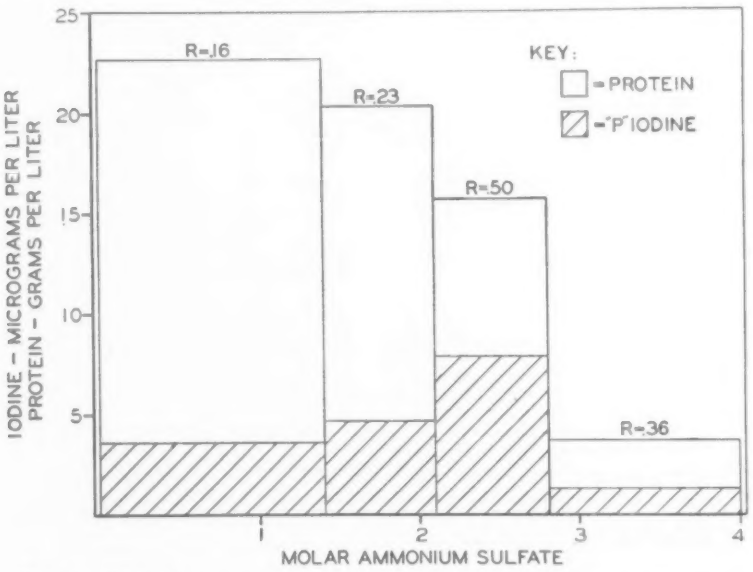

FIG. 2.—When plasma protein fractions are precipitated *seriatim* at increasing concentrations of ammonium sulphate, the natural protein-bound iodine is found to reside prominently in the traditional albumin fraction. The results illustrated are for horse serum.

The results shown in Figure 2 were obtained with horse serum. It is evident that the globulin fractions precipitated by a molarity of 1.6 contain little protein-bound iodine. Slightly more appears in the upper globulin fractions, which are precipitated by a molarity of 2.1. The bulk of the protein-bound iodine, however, is associated with the "crude" albumin fractions precipitated below a molarity of 3.0. The proteins soluble in that concentration of ammonium sulphate did not

* M. = molar.

contain an important amount of protein-bound iodine. The ratio of iodine to protein (micrograms of iodine per gram of protein) rose successively to a maximum value of 0.8 in the albumin fraction soluble at 2.4 and insoluble at 2.8, *i.e.*, in a rather sharply defined fraction. The crystalbumin fraction, however, contained relatively little protein-bound iodine.

Similar studies on human plasma have yielded comparable results. These are reported in detail elsewhere.[17] The total plasma protein-bound iodine in a sample of pooled human plasma was found to be 5.2 μg. per 100 cc. In this instance the crude proteins had been precipitated and prepared as a dry powder. For comparison, in a series of 10 normal control individuals the plasma protein-bound iodine values ranged from 4.6 to 5.9 μg. per 100 cc., as determined by the technique described earlier in this report; their mean was 5.2.

It should be noted that from a strict physicochemical standpoint, the partially purified protein fractions illustrated in the figure are still crude, even though they represent a considerable technical advance over the older, traditional method of separation which continues to be described in the literature.[18] In other words, it is conceivable that a small amount of specialized iodoglobulin may be responsible for the high iodine content found in our crude "albumin" fractions. It must be noted, however, that in a preparation of human albumin which appeared to be 100 per cent albumin by electrophoresis measurements, a very high iodine concentration was found.

This last-mentioned material was prepared by Dr. S. H. Armstrong, Jr. in the laboratory of Prof. E. J. Cohn. A detailed account of these observations will be found elsewhere,[17] but a brief summary may be given to illustrate the general procedure. Iodine was determined in the several crude protein fractions separated from whole human plasma by ethanol under special precautions. Per gram of protein, the iodine content in micrograms of these fractions was as follows: I, fibrinogen 0.2; II, first globulin fraction 0.2; III, intermediate globulin fraction 0.3; IV, alpha and beta globulins 1.2; V, main albumin fraction 0.9; VI, residual albumin, 0.8. These data reflect those obtained with ammonium sulphate in that 58% of the total protein-bound iodine appeared in the main albumin fraction, V, as compared with 46% in the ammonium sulphate separation; but the highest ratio appeared in the fraction containing alpha and beta globulins. When Fractions IV and V were purified further, the ratios were as follows: for α-β globulins 1.7; for albumin 1.4. These last two values are especially significant because electrophoretic measurements showed the α-β globulins to contain less than 5% of albumin and the albumin fraction to contain no perceptible globulin. In short, even after rather careful precautions to obtain homogeneous α-β globulin and albumin fractions, respectively, high concentrations of iodine were found in

both. Possibly the former contained slightly more iodine per gram of protein, but considerably less in terms of the total mass of protein.

Collateral observations indicate that there are several albumins in human plasma, and that the iodine is preferentially allocated to one or more of these fractions.

Relationship of "P" Iodine to "I" Iodine. As described above, after iodine medication or other contact with iodine-containing material, the plasma iodine is increased. When the inorganic iodine rises far above normal, the apparent protein-bound iodine also is elevated. As demonstrated in Figure 3, the protein-bound

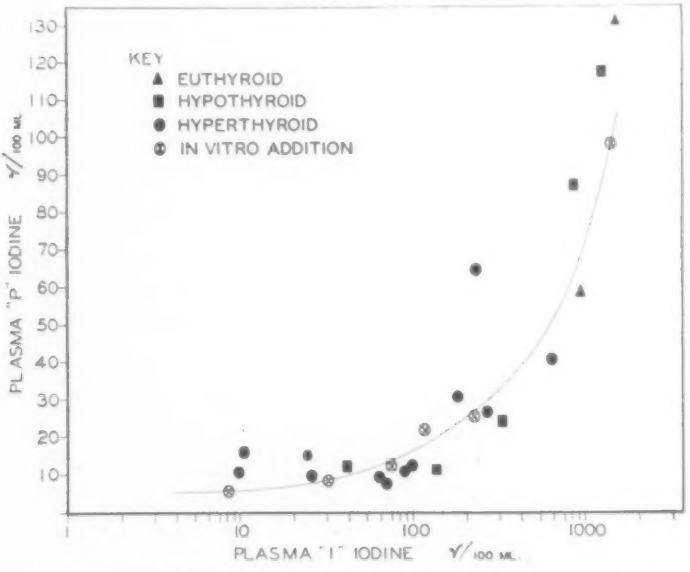

FIG. 3.—A spurious elevation of the protein-bound "P" iodine occurs when the inorganic "I" iodine is unusually high, as described in the text.

iodine, "P," is roughly proportional to the inorganic iodine value, "I," determined simultaneously. This is true irrespective of the state of thyroid function, although, as already mentioned, there is some tendency for hyperthyroid individuals to show lower levels of inorganic iodine than do normal or myxedematous patients on comparable doses of iodide, and hence a lower "P" iodine value.

This elevation of protein-bound iodine which accompanies elevation of the inorganic fraction can be duplicated *in vitro* by the addition of potassium iodide in increasing amounts to normal human plasma. Such an experiment is illustrated in Figure 3. As indicated by the smooth curve, the values obtained *in vitro* are

entirely comparable with the values determined for random samples of blood from patients receiving therapeutic doses of iodine.

The mechanism by which this spurious elevation of plasma "P" iodine occurs is not clear. It may be due to occlusion or adsorption, and thus might be regarded as a chemical artefact. In any case, it constitutes a technical hazard which must be avoided in evaluating levels of plasma "P" iodine. If the inorganic "I" iodine is found to be unusually high, the plasma "P" iodine value must be considered as meaningless, at least for the present.

Response of Plasma Iodine to Thyroxine Therapy. When crystalline thyroxine in solution is administered to a patient, a persistent rise in the plasma "P" iodine value occurs. This effect is illustrated by a case of exophthalmic ophthalmoplegia[3] in a male of 36 years, whose exophthalmos receded strikingly during treatment with thyroxine. Before and during therapy, basal metabolic rate and "P" iodine values were determined. These data were as follows:

Date.	mg. Thyroxine therapy.	B. M. R.	Plasma. "P" iodine (μg. per 100 cc.).	Plasma. "I" iodine (μg. per 100 cc.).	Exophthalmos by Luedde ophthalmometer (mm.) O.D.	Exophthalmos by Luedde ophthalmometer (mm.) O.S.
2/13	None	−10	5.6	..	24	23.5
4/7 to 4/11 inc.	1 mg. i.v. daily	−5	10.3	4.9		
4/12 to 4/15 inc.	1 mg. i.v. daily	−8				
4/16	10 mg. i.v.					
4/17	10 mg. i.v.					
4/18 to 4/22 inc.	None	+34	18.0	3.9		
5/1	...	..	..	..	18	17.5
5/28	...	..	..	..	18	18.5

Relationship of "P" Iodine to Thyroid Status. As illustrated in Figure 1, the plasma protein-bound "P" iodine tends to vary directly with the basal metabolic rate or possibly as the antilogarithm of the basal caloric consumption. The values in hypothyroidism may be less than 1 mg. per 100 cc. and the mean is 2 mg. per 100 cc.; in euthyroidism the levels range between 4 and 8 mg. per 100 cc.; and in hyperthyroidism the values exceed 8 mg. per 100 cc. and may rise as high as 25 mg. per 100 cc. A detailed study of "P" iodine in thyroid disease is presented elswehere[15] and in the companion report immediately following this communication.

Fractions of Protein-bound Iodine Resembling, Respectively, Thyroxine and Diiodotyrosine. Elmer, Luczýnski, Rychlik and Schepps[4] obtained large quantities of human blood pooled from different patients and hydrolyzed it after the manner of Leland and Foster.[9] They estimated that approximately 40 to 60% of the "organic" iodine in normal blood behaved like thyroxine. Similar values were obtained in blood from hyperthyroid individuals. Likewise Trevorrow[20] reported that about one-third of the protein-

bound iodine in the plasma of animals had properties like thyroxine, whereas the remainder behaved like diiodotyrosine.

In the present studies these "T" and "D" fractions were estimated in the following fashion:

Proteins were precipitated from 10 cc. of plasma, as described previously, and then suspended in 10 cc. of water to which had been added approximately 20 mg. of commercial pepsin (Merck), 0.5 cc. of 18 N sulphuric acid and 5 drops thymol blue. This mixture was digested at 37° C. in a 50-cc. centrifuge tube until the contents were liquid; *i. e.*, for 12 to 24 hours. Acid was added as necessary to keep the digest at pH 1.5. After digestion the pH was raised to 2.5 and the mixture extracted with a volume of butyl alcohol equivalent to twice the volume of plasma originally used. Centri-

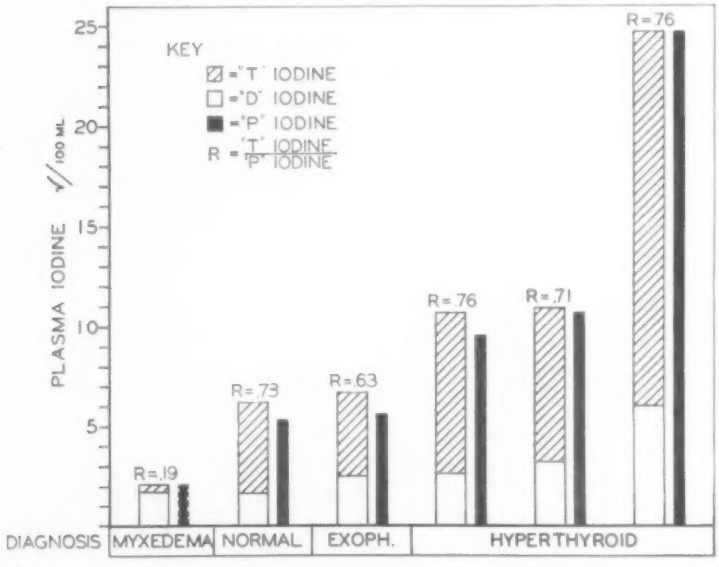

FIG. 4.—In cases ranging from myxedema to marked hyperthyroidism, the absolute rise in protein-bound "P" iodine is due largely to an elevated thyroxine-like "T" fraction.

fugation at 3000 r.p.m. separated the solutions. A gel frequently formed in the butyl alcohol layer, but after being broken up by jarring or with a stirring rod, centrifugation reduced it to a small precipitate located at the interphase. The butyl alcohol layer was then withdrawn and the water layer was re-extracted with one-half the volume of butyl alcohol used before. After centrifugation this butyl alcohol layer was added to the first butyl alcohol extract. Any precipitate which remained at the interface was carried along with the butyl alcohol. The water layer which remained was discarded. The combined butyl alcohol extracts were shaken with an equal volume of 2 N sodium hydroxide containing 5% sodium carbonate in separatory funnels which could be centrifuged in special trunnion carriers at 1000 r.p.m. After this centrifugation the alkaline layer was removed and the butyl alcohol layer reëxtracted with one-half the original volume of alkali. After another centrifugation the alkaline layer was withdrawn

and added to the first alkaline extract. This alkaline aqueous solution was designated as the D-fraction and was analyzed for its total iodine content.

The butyl alcohol layer was evaporated to dryness and 25 cc. of water was added. This solution was designated as the T-fraction and was analyzed for its total iodine content.

As shown in Figure 4, illustrating a series of plasma values from patients with myxedema and hyperthyroidism as well as from normal individuals, the D-fraction represents about 30% of the "P" value; in absolute terms this varies from 1 to 6 mg. per 100 cc. In normal individuals the T-fraction is approximately twice the D-fraction. This T-fraction, however, varies with the thyroid status of the individual, tending to be practically absent in myxedema and excessively large in severe hyperthyroidism. Indeed, the marked variations of the protein-bound iodine with varying physiologic thyroid status, to be discussed in the subsequent paper, are probably in large measure due to changes in the T moiety.

The efficiency of the method in recovering thyroxine, when added to water as well as to human plasma, is demonstrated by the data in Table 2. When thyroxine is added to plasma, not only is it recovered entirely as protein-bound iodine, as reported also by Trevorrow,[20] but it contributes exclusively to the "T" fraction.

TABLE 2.—THYROXINE–LIKE AND DIIODOTYROSINE–LIKE COMPONENTS OF PROTEIN-BOUND "P" IODINE.

Solvent.	Addition.	Protein-bound "P."	Thyroxine-like "T."	Diiodotyrosine-like "D."
Water . . .	0.65 μg. per 100 cc. thyroxine	..	0.5 μg. per 100 cc. (75% recovery)	
Water . . .	0.59 μg. per 100 cc. diiodotyrosine	..	..	0.54 μg. per 100 cc. (92% recovery)
Human plasma	None	6.0	5.5	1.5
Human plasma	5.6 μg. per 100 cc. thyroxine	11.9	11.2	2.0

Discussion. It should be emphasized that the methods described here have been simplified for clinical use, and may not be universally applicable to problems of a more fundamental type. For example, all of the plasma proteins have been precipitated routinely, whereas the naturally circulating iodine resides prominently in the crude albumin range. The chief precautions which have proved essential to uniformity of results have been: 1, overnight fasting; 2, the use of plasma instead of whole blood; 3, the elimination of incoagulable, "inorganic" iodine; and 4, the exclusion of unusual contamination from iodine-containing material.

As described, the method *in toto* requires careful work but no complicated apparatus. As the microdetermination of iodine becomes further improved and simplified, it seems likely that this

general procedure may become a routine test in large clinics which deal with endocrinologic problems.

At present, the method is not applicable without special precautions when gross contamination with iodine has occurred within 3 weeks. Routine therapy with Lugol's solution, diodrast kidney clearance tests, cholecystography or the painting of iodine upon the skin represent hazards which vitiate the "P" iodine determination. In case of doubt, the "I" iodine should be determined simultaneously, and if it is above 5 or 6 μg. per 100 cc., the "P" iodine value should be viewed with distrust.

Too great stress should not be placed upon the absolute values for iodine concentration stated here. Such values are relative and depend upon the technique involved. Estimations of the apparent iodine concentration of the blood, as reported from various laboratories, have been falling steadily for two decades, and may still vary somewhat in different laboratories. Fortunately, however, the reported range of normal values has become rather stable in the past few years. Each institute should learn and state clearly its own range for normal values.

Within these limitations, the determination of "P" iodine appears to be a practical and useful method for clinical investigation and routine diagnosis, because it affords at least a roughly quantitative measurement of the circulating thyroid hormone. As endocrinology rapidly is becoming a subdivision of the medical sciences that is being studied quantitatively, the need for such precise measurements of hormone concentration in biological and clinical material is obvious.

Summary. The protein-bound iodine in the blood plasma of man and of animals was found to reside chiefly in the traditional albumin fraction. This iodine was subject to fluctuations dependent upon thyroid activity. Such fluctuations, in absolute terms, were due chiefly to a thyroxine-like moiety thereof. The moiety resembling diiodotyrosine might increase proportionately, but it contributed relatively little to the absolute change from the normal level. Accordingly, total protein-bound iodine fluctuated largely with thyroxine-like iodine. In myxedema, the thyroxine-like fraction practically disappeared.

Despite variations in this protein-bound iodine, the ionized iodine (inorganic) appeared to be rather low and approximately constant under the conditions of our study, regardless of the state of thyroid activity. Nevertheless, it was found to increase markedly when extraordinary, though perhaps very small, amounts of iodine entered the organism. Simultaneously a false increase in the protein-bound iodine occurred which could be reproduced by the addition of iodide to plasma *in vitro*.

The results indicate that the protein-bound thyroxine-like moiety of the plasma iodine is a good objective index of circulating thyroid

hormone. They suggest, also, that plasma protein-bound iodine might be used clinically to confirm physiologic thyroid status. This application to cases of thyroid disease will be described in the communication immediately following.

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PROTEIN-BOUND IODINE IN BLOOD.

VI. Its Relation to Thyroid Function in 100 Clinical Cases.*

By William T. Salter, M.D.,

Assistant Professor of Medicine, Harvard Medical School; Associate Physician, Thorndike Memorial Laboratory; Junior Visiting Physician, Boston City Hospital,

A. Merton Bassett, M.D.,

Research Fellow, Harvard Medical School; Assistant Resident Physician, Thorndike Memorial Laboratory; Assistant in Medicine, Boston City Hospital,

and

Thomas S. Sappington, M.D.,

With the technical assistance of Sophia M. Simmons, S.B.

Boston, Mass.

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.)

In the preceding communication[1] it was suggested that the protein-bound iodine of blood plasma might be used as an index of

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the circulating thyroid hormone. In this way a quantitative estimate of effective thyroid activity might be gained. It is the purpose of the present report to describe the results so obtained in 100 clinical cases.

Inasmuch as the significance of the blood iodine has been discussed in detail elsewhere,[18] only a few outstanding examples of recent clinical studies will be cited. As regards total blood iodine, important series of cases have been reported by Perkin and Lahey,[14] by Turner, DeLamater and Province[19] and by Riggs, Gildea, Man and Peters.[17] Perkin and Hurxthal[13] and McClendon, Foster and Kirkland[8] have also studied the so-called "organic" iodine fraction as determined by separation with organic solvents like alcohol and acetone. In general, all of these series, one of which comprises over 1000 cases,[14] show a distinct tendency on the part of the blood iodine level to vary directly with thyroid activity. In all, however, with the partial exception of the recent series of Riggs and his associates,[17] certain grave difficulties in interpretation have been encountered. For example, in myxedema the values have often fallen within a low normal range, as pointed out recently by Greene.[5] Likewise in the large series reported by Perkin, Lahey and Cattell[15] many cases of Graves' disease showed a normal blood iodine concentration and a smaller percentage of normal individuals showed a high blood iodine value. In fact, Perkin and Cattell[12] have emphasized this discrepancy as an aid in deciding upon the therapeutic procedure to be employed.

Because of these discrepancies, *plasma protein-bound iodine* has been determined in 100 cases of suspected thyroid disturbance, in the hope that careful scrutiny of a relatively small number of cases might reveal correlations and reasons for lack of correlation.

Methods. Blood for the determination of plasma protein-bound iodine was drawn after overnight fasting and, whenever possible, immediately following determination of the basal metabolism. Special precaution was taken to be sure that no thyroid or iodine therapy had been used within the preceding 3 weeks, and the latter therapy was excluded in doubtful cases by a simultaneous determination of the plasma ("inorganic") iodide. The chemical methods used have been described in detail in previous communications.[1,18]

The basal metabolic rates were determined with the Benedict-Roth apparatus under the usual standard conditions as described by Dubois and Benedict. The normal standards employed were those of Dubois as prepared by Boothby and Sandiford at the Mayo Clinic.

Classification of Cases Studied. For euthyroid controls, 10 individuals were selected, half of them healthy laboratory workers and the other half patients in whom no suspicion of thyroid disturbance was entertained.

Each of the 100 test cases received individual evaluation and was classified on the basis of: *1*, ultimate clinical diagnosis; *2*, ultimate or most trustworthy basal metabolic rate, and *3*, microscopic appearance of the thyroid gland, when available. This classi-

fication was made without knowledge of the plasma iodine concentration. It was thus possible to distribute the 100 cases into four main clinical subgroups; *i.e.*, hypothyroid, euthyroid,* hyperthyroid and ophthalmic cases (Table 1). The latter group consisted of so-called "Graves' disease without hyperthyroidism"[10] or "exophthalmic ophthalmoplegia."[2] This latter syndrome is discussed in detail below. For future reference, a census of the cases studied has been appended.

TABLE 1.—DISTRIBUTION OF CASES STUDIED.

Diagnosis.	No. of cases.	B.M.R.	"P" iodine vs. diagnosis: Compatible.	"P" iodine vs. diagnosis: Incompatible.
Series I. Compatible Diagnosis and B.M.R.				
A. Hypothyroid	20	Low	20	0
B. Euthyroid	5	Standard	5	0
C. Hyperthyroid	38	High	37	1
D. Ophthalmic	8	Standard	8	0
Total	71		70	1
Series II. Incompatible Diagnosis and B.M.R.				
A. Euthyroid	9	Low	9	0
B. Euthyroid	12	High	12	0
C. Ophthalmic	5	Low	4	1
D. Ophthalmic	2	High	2	0
E. Hyperthyroid	1	Standard	1	0
Total	29		28	1
Grand total	100		98	2
Control Series				
Euthyroid	10	Standard	10	0

From such a classification certain interesting correlations appeared which served as the basis for further analysis. Nearly all the cases adjudged euthyroid had plasma protein-bound iodine values greater than 3.9 μg. per 100 cc. and less than 8.1 μg. per 100 cc. (see Figs. 1 and 2), and these figures were selected arbitrarily as the limiting values of the euthyroid state. The criteria for the basal metabolic rate used here were as follows: Less than 10% was considered to be substandard, from −10 to +15%, inclusive, was called normal and greater than 15% was called elevated. It was also apparent that evaluation of the plasma protein-bound "P" iodine would be facilitated by regrouping the 100 cases into two series, as follows: On the basis that for each case, one of the above described clinical diagnoses and the truest estimate of basal metabolic rate were either compatible or incompatible, the cases were segregated into Series I and Series II, respectively. For example, a case of obvious myxedema with a basal metabolic rate of −40

* A euthyroid individual is one whose tissues at large receive the normal amount of thyroid hormone daily, irrespective of the anatomic state of the thyroid gland.

was placed in Series I. On the contrary, a case diagnosed clinically as euthyroid with a basal metabolic rate of +40 (before iodine or other therapy) was placed in Series II. These two series are referred to as the "compatible" Series I and the "incompatible" Series II, respectively.

Obviously, Series II requires the greater scrutiny. The standard diagnoses were therefore subdivided further into subgroups (A, B, C, D and E) according to the nature of the discrepancy involved (Table 1). Typical and representative cases in each of the subgroups will be presented later.

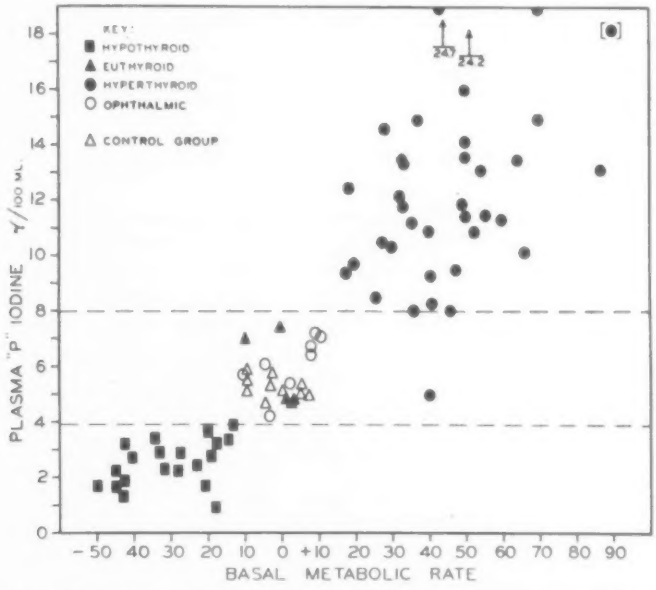

FIG. 1.—In two-thirds of the test cases studied, the clinical diagnosis and the basal metabolic rate were compatible, as shown in the figure. Plasma protein-bound iodine values are given for these cases and for 10 euthyroid controls. The "ophthalmic" cases are described in the text.

On the basis of these classifications and with the arbitrary standards for plasma protein-bound iodine and basal metabolic rate stated, it was possible to compare the clinical, metabolic and blood iodine findings in order to ascertain the significance of the latter.

Analysis of Cases by Clinical Groups. SERIES I: CLINICAL DIAGNOSIS AND BASAL METABOLIC RATE COMPATIBLE. This group, illustrated in Figure 1, comprises 71 cases, or 71% of the total number.

Hypothyroid. As shown in Figure 1, 20 cases of hypothyroidism were included in this category and all had plasma protein-bound

iodine values of less than 4 μg. per 100 cc., *i.e.*, definitely subnormal. This suggests that plasma protein-bound iodine is of distinct value in confirming the diagnosis of poor thyroid function, particularly in those cases in which full-blown myxedema is not present. Heretofore such cases often have been indistinguishable from the normal when whole blood iodine[5] or "organic" iodine[6] has been measured.

Controls. As previously stated, 10 euthyroid controls were added to the series of 100 test cases. As shown in Figure 1, all 10 had plasma protein-bound iodine values greater than 4 μg. per 100 cc. and less than 8 μg. per 100 cc. In this way "normal" values were defined.

Euthyroid Cases. Likewise 5 cases, who at first were suspected of thyroid disease despite normal basal metabolic rates but who were diagnosed finally as euthyroid, all had "normal" plasma protein-bound iodine values.

Hyperthyroid. In Figure 1 are charted also 38 cases of hyperthyroidism. All but one had plasma protein-bound iodine values exceeding 8.0 μg. per 100 cc. On the whole, the elevation of the iodine level tended to be proportional to the elevation of the basal metabolic rate, but individual exceptions were not infrequent. It should be stressed that in each of the above cases of hyperthyroidism, *at the bedside* it was decided that the patient showed sufficient evidence of both (*1*) neurologic and (*2*) calorigenic disturbance to warrant the diagnosis of Graves' disease with hyperthyroidism.

Ophthalmic. The ophthalmic cases include the syndrome defined by Means[11] and Salter[18] as "Graves' disease without hyperthyroidism." These authors have noted the absence of calorigenic disturbances (warm, moist skin; excessive sweating and weight loss) in cases which otherwise resemble classical Graves' disease. Accordingly, they suggest that despite the neurologic symptoms, such cases are in effect euthyroid and have basal metabolic rates which usually are normal or substandard. In addition, the electroencephalogram may show normal alpha cortical rhythm and reaction time.[3] The majority of these cases exhibit marked exophthalmos and have been described by Brain[2] as "exophthalmic ophthalmoplegia." Occasionally cases of malignant exophthalmos display clinical and metabolic evidence indicating that they are hyperthyroid or hypothyroid, and in this report are classified as such; in other words, the classification "ophthalmic" has been reserved only for those cases adjudged to be euthyroid.

Eight ophthalmic cases are represented in Figure 1; all had normal basal metabolic rates and all had normal plasma protein-bound "P" iodine values. A representative case history follows.

CASE 66.—B. H. (B. C. H. No. 1017062), a 36-year-old male stationary engineer entered the hospital, complaining of bilateral prominence of his

eyes and conjunctivitis of 7 months' standing. There was no change in appetite nor loss of weight. There was no goiter, tremor or tachycardia. The skin was normal. The basal metabolic rate was −10%. Measurement of the eyeballs with the Luedde exophthalmometer[7] showed the cornea to be 24 mm. anterior to the lateral orbital margin. Plasma protein-bound iodine was 6.4 μg. per 100 cc. After 3 weeks of therapy with large doses of thyroxine the basal metabolic rate rose to +34% and the plasma protein-bound "P" iodine to 18 μg. per 100 cc. During this time the eyeballs receded 7 mm. and the chemosis and inflammation subsided.

The favorable course of the exophthalmos when the circulating plasma iodine was artifically elevated is, of course, consistent with the thesis that Graves' disease and physiologic hyperthyroidism may be dissociated. Indeed, although there is still considerable dispute as to the appropriate therapy in these cases, recent publications[4,11] have suggested that thyroxine or thyroid medication is superior to thyroidectomy, which may accentuate the exophthalmos to an alarming degree.

In addition to idiopathic cases of exophthalmic ophthalmoplegia, the "ophthalmic" group here reported includes both patients who have submitted to thyroidectomy and also chronic cases of physiologic hyperthyroidism which have "burned out." Because cases of Graves' disease apparently may show all intergrades of thyroid activity, it is obvious that the selection of the euthyroid cases taxes clinical judgment to the point of arbitrary decision. As will appear presently, however, such crude judgments have sufficed for present purposes.

In summary, the over-all correlation of basal metabolic rate with plasma protein-bound iodine in Series I is very high, namely, 0.82 ± 0.05 for the 71 cases plus the 10 controls, totaling 81 individuals. The statistical P-value is less than 0.001. As the cases in this series are quite straightforward as regards their diagnosis, there is little need to discuss them further. There remain, however, 29% of the cases which must be classified otherwise.

Series II: Clinical Diagnosis and Basal Metabolic Rate Incompatible. This series consists almost entirely of individuals who were adjudged euthyroid on general clinical grounds, but who showed an abnormal basal metabolic rate. In certain cases it might be surmised that the recorded metabolism was not truly basal, but it was the best value obtainable after repetition under routine conditions in a large hospital. These cases are recorded as Series II in Table 1 and they are charted in Figure 2. Characteristics and representative cases of each of these subdivisions (see Table 1) follow.

Series II-A: Euthyroid Individuals With Substandard Basal Metabolic Rate. In this subgroup are included 9 cases with a basal metabolic rate below −10, in whom no definite clinical evidence of hypothyroidism could be discovered. It is concluded that these individuals represent cases of a substandard but physio-

logically normal basal metabolic rate. In 2 cases the diagnosis was hypopituitarism with secondary hypogonadism. Despite basal metabolic rates of −20 and −30%, respectively, their lack of clinical evidence of hypothyroidism and their negligible response to thyroid medication seemed to warrant a diagnosis of euthyroidism. All of these cases had normal plasma protein-bound iodine values. A representative case follows in summary.

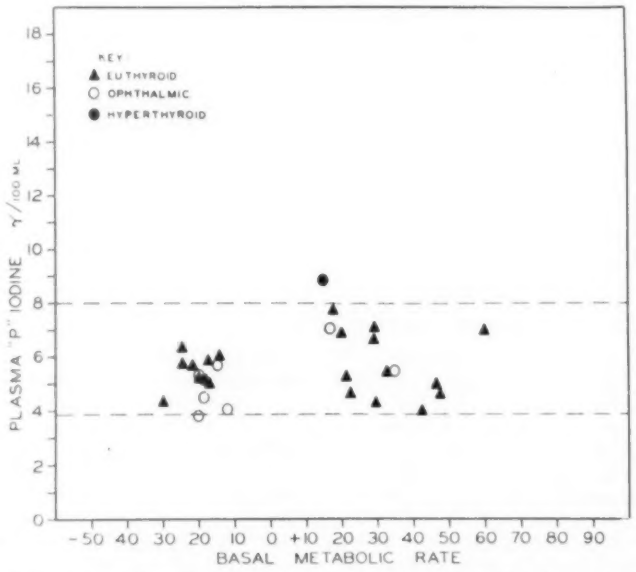

FIG. 2.—In approximately one-third of the test cases the basal metabolic rate determination was incompatible with the final clinical diagnosis. Most of these cases had a normal plasma protein-bound iodine, as shown in the figure. The "ophthalmic" cases are described in the text.

CASE 75.—J. O'S. (B. C. H. No. 1004320), a 36-year-old male clerk, entered the Boston City Hospital complaining of nervousness, sweating, insomnia and loss of weight. He appeared very nervous, had a constant tachycardia and a slight manual tremor. His thyroid was of normal size and his skin, though of fine texture and moist, was quite cool. Hyperthyroidism was suspected at first but his basal metabolic rate was −22%. Certain psychic problems were uncovered and when corrective measures were instituted marked symptomatic relief followed.

SERIES II-B: EUTHYROID INDIVIDUALS WITH ELEVATED BASAL METABOLIC RATE. This group of 12 cases comprised patients suffering from cardiac decompensation, neurasthenia or hyperpyrexia, in whom the possibility of hyperthyroidism was considered at first on general clinical grounds and apparently confirmed by the finding of an elevated basal metabolic rate. In these cases, however, relief of the underlying condition, *e. g.*, circulatory failure

or anxiety, sufficed to return the basal metabolic rate to normal without specific iodine therapy. In the few cases in which a therapeutic trial of iodine alone was made, it proved ineffective in correcting the symptoms or in lowering the elevated metabolic rate. Thus the course of the illness caused abandonment of the initial impression of hyperthyroidism. All the patients in this group had normal plasma protein-bound iodine levels. A representative case follows.

Case 86.—P. McK. (B. C. H. No. 967590), a 52-year-old policeman, entered the hospital complaining of dyspnea and edema of the ankles. The blood pressure was 190/110 mm. Hg. Hyperthyroidism was suspected because of restlessness, staring eyes and pink complexion of the face. Attempted basal metabolic rate readings yielded approximately +40% on repeated determinations. After several weeks of rest in bed and treatment with digitalis, the initial symptoms of anxiety abated as the circulation improved and the basal metabolic rate became normal.

Series II-C: Ophthalmic Cases With Substandard Basal Metabolic Rate. This group of 5 cases was composed of patients who, as described above, presented marked exophthalmos and, in some cases, neurologic symptoms of Graves' disease, but no disturbance of caloric balance. They were all considered to be euthyroid despite substandard basal metabolic rate. A characteristic case follows.

Case 95.—M. C. (B. C. H. No. 1028107), a 39-year-old American housewife, entered the hospital with a history of diplopia, vertigo and sudden exophthalmos of 1 month's duration. Two years previously she had a thyroidectomy for typical thyrotoxicosis, and thereafter until her recent illness had been in excellent health. Examination disclosed a well nourished woman with marked exophthalmos, lid lag and convergence defect. Moderate periorbital edema was present. Her speech and movement were quick and nervous. The skin was of normal texture and temperature, but dry. Her heart rate was normal and she had no tremor. Basal metabolic rate was −20%.

All but one of these cases had normal plasma protein-bound "P" iodine values. The exception occurred in an ophthalmic case which had developed following thyroidectomy for thyrotoxicosis. The basal metabolic rate was −20%. Although mild hypothyroidism was suspected at first, the patient was finally judged to be euthyroid. His plasma protein-bound iodine value was barely subnormal (3.9 μg. per 100 cc.).

Series II-D: Ophthalmic Cases With High Basal Metabolic Rate. Cases so classified present peculiar difficulties in diagnosis. Obviously the designation of clinical euthyroidism is challenged by the presence of neurologic symptoms, exophthalmos and elevated basal (?) metabolic rate which may characterize these patients in varying degree. In such borderline cases two equally experienced clinicians may disagree at the bedside as to the evidence for or against normal or increased metabolism. Fortunately there were

only 2 such cases in the series. Because the clinical evidence for abnormally high caloric consumption was unconvincing, they were classified as euthyroid, without knowledge of the plasma iodine concentration. The problems in diagnosis and the evidence for adjudging them euthyroid are well exemplified in the following case.

Case 99.—M. B. (B. C. H. No. 990460), a 41-year-old widow entered the hospital complaining of sciatica and nervousness. A diagnosis of hypertensive cardiovascular disease and anxiety state was made. She reëntered several months later, complaining of increasing irritability, excessive sweating when nervous, and palpitation. Her cardiovascular status was unchanged except for the presence of tachycardia; her thyroid was normal to physical examination, and although she had slight exophthalmos no associated eye signs were present. Her initial basal metabolic rate was +36%. After this single determination of metabolic rate the patient received iodine therapy, following which her apparent basal metabolic rate fell to +9% without coincident improvement in symptoms. During the next few months she took iodine intermittently at home, and on her third entry presented essentially the same findings as on previous discharge. Again the metabolic rate was elevated and again simultaneous bed rest, reassurance and iodine caused it to return to normal. Thyroidectomy was performed and the pathologic diagnosis was reported by one pathologist as: colloid goiter; by Dr. Shields Warren as: longstanding hyperinvolution of old primary hyperplasia—not the result of recent iodine medication.

Despite her subtotal thyroidectomy the patient experienced no relief from her complaints of nervousness, tachycardia and sweating, for which she was hospitalized again 9 months after her operation. Physical findings, except for absent thyroid gland, were unchanged from her previous entries and again the basal metabolic rate was +40%. This time, however, iodine was withheld and on bed rest alone the basal metabolic rate returned to normal. Prior to her thyroidectomy, at a time when the basal metabolic rate had been +36%, her plasma protein-bound iodine level was normal (5.5 μg. per 100 cc.), and therefore consistent with the diagnosis one would be forced to make in retrospect, *i. e.*, Graves' disease *without* hyperthyroidism.

The other case in this group also had a normal plasma protein-bound iodine value.

Series II-E: Hyperthyroidism With Normal Basal Metabolic Rate. This category is represented by one case of recurrent hyperthyroidism, recognized clinically as "mildly toxic," in which the basal metabolic rate was +14%. It falls, therefore, into the incompatible group, although obviously the estimation of physiologic status was a borderline decision. Interestingly enough, the plasma protein-bound iodine was only slightly above the normal limit, *i. e.*, 8.8 μg. per 100 cc.

Comment. Of the cases shown in Figure 2, those in Series II-A, II-B, II-C and II-D may properly be considered to be euthyroid on clinical grounds, and in all but one of these cases the plasma protein-bound iodine was actually normal. It should be remarked that this second large series, if combined with the first series, would distort the correlation between the basal metabolic rate and plasma iodine determination as indices of abnormal thyroid function. In

fact, such a combination might lead to the same conclusion that previous series have led to, namely, that a poor correlation exists between basal metabolic rate and blood iodine concentration. In the present instance, however, the coefficient of correlation for the entire 100 cases would be reduced only to 0.79 ± 0.04.

Recrudescent Hyperthyroidism. In order to evaluate a biochemical procedure like plasma protein-bound iodine, it is obviously necessary to crowd one's test cases into a simplified rigid scheme of classification as regards diagnosis. In this process, difficult diagnostic situations occasionally arise which require very arbitrary treatment. For example, such a case may be in spontaneous remission when first studied, and may later show clinical evidence of excessive caloric turnover, together with an elevated basal metabolic rate and high plasma iodine level. A synopsis of such a case follows.

CASE 83.—M. N. (B. C. H. No. 965392), a 44-year-old Greek housewife, entered the hospital complaining of palpitation, shortness of breath and edema of the ankles of several months' duration. Examination showed a thin, anxious woman in obvious congestive failure without auricular fibrillation. There was no goiter, tremor or marked exophthalmos. Blood pressure was 160/95. After several weeks of treatment with digitalis and rest, cardiac compensation was restored. The basal metabolic rate, however, remained close to +40% and the pulse over 80. Plasma protein-bound iodine was 6 μg. per 100 cc. A therapeutic test with iodine did not alter the basal metabolic rate, which was determined with some difficulty because of the patient's apprehension and mild orthopnea. The patient was discharged on iodine therapy and given a course of Roentgen ray treatments to the thyroid region. Ten months later she reëntered, presenting much the same picture as at the previous admission. At this entry, however, the thyroid was definitely palpable, a little more prominent on the right, and the thyroid isthmus seemed hyperplastic. After restoration of cardiac compensation the basal metabolic rate rose steadily from +30% to +55% in 3 weeks. At this time the plasma protein-bound iodine was found to be 10 μg. per 100 cc. Routine treatment with Lugol's solution was begun and in 2 weeks the basal metabolic rate had fallen to +25%. A clinical diagnosis of toxic nodular goiter was made.

The question might be raised by some clinicians whether this case was originally one of "burned out" hyperthyroidism in which iodine therapy had supplied new building material wherewith the thyroid gland could manufacture again an excess of hormone. In other words, the mechanism involved would resemble that of "Jod Basedow" which has been much discussed in Europe.[9,16]

This case is presented to emphasize the fact that the determination of plasma protein-bound iodine *at one instant* can reflect only the hormonal activity at that time. It can not, if normal, guarantee against future hyperthyroidism, either fresh or recrudescent. Moreover, it is not an automatic arbiter of therapeutic procedures. Indeed, in Case 91, clinically adjudged euthyroid as shown in the appended census of cases, a hyperplastic gland was found at postmortem examination. Did premature therapy with iodine contribute to the cardiac embarrassment? Such cases as these raise

problems which suggest that smoldering or latent hyperthyroidism should be studied anew from the standpoint of therapy.

Plasma Iodine Fractions, Inorganic and Protein Bound. In Figure 3 are given data for 2 individuals, one suffering from classical myxedema and the other normal. By chance the circulating inorganic "I" iodine in the myxedema case was a little greater than the average and, on the other hand, the similar fraction for the normal individual was a little lower than the average. In consequence of these fortuitous fluctuations the total iodine value for the myxedema case was 7.1 μg. per 100 cc. whereas the similar value for the normal individual was only 6.3. When the protein-bound "P" fractions were determined, however, that for the myxedematous patient was only 2.2, whereas the normal individual showed 4.8 μg. per 100 cc. As explained in the preceding communication,[1] no great significance is attached to the absolute values yielded by a

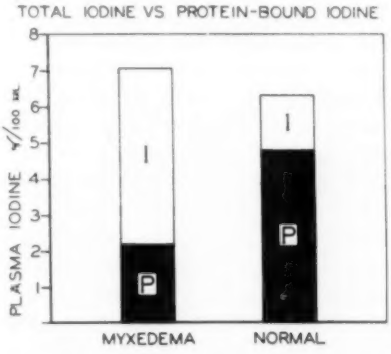

FIG. 3.—Values are given for inorganic "I" iodine and protein-bound "P" iodine in the plasma of two individuals. Obviously, unless the inorganic iodine is excluded the significant variations in "P" iodine may be masked.

partially empirical technique; the relative magnitude only is important. These two instances are cited in contrast to each other in order to illustrate why the determination of total plasma iodine may be inadequate for diagnostic purposes. This finding in part explains why, as pointed out by Greene,[5] it has not been easy heretofore to distinguish hypothyroidism from euthyroidism on the basis of blood iodine analysis. On the other hand, all 20 of the cases of clinical hypothyroidism described in this report showed abnormally low levels for plasma protein-bound iodine.

Discussion. The data presented here indicate that when a final diagnosis of suspected thyroid disease is made in a large teaching clinic and confirmed by the determination of basal metabolic rate, the general range of plasma protein-bound iodine can be predicted with a considerable degree of certainty. In most of such cases, therefore, the analysis would be only of mild confirmatory interest.

In about one-third of the cases encountered in a large clinic, however, in which thyroid status has been suspected, the basal metabolic rate has proved not to be compatible with the best bedside diagnosis. In this second category a determination of protein-bound iodine has usually given a value confirming the clinical diagnosis and the basal metabolic rate has turned out to be misleading. In these latter cases, therefore, the determination of plasma iodine could facilitate earlier arrival at a diagnosis. This was particularly true when the elevation in basal metabolic rate was due to cardiac decompensation.

In mild hypothyroidism, before frank myxedema has developed, the finding of a low plasma protein-bound iodine has proved of considerable value in establishing the need for thyroid therapy. The application of this test is particularly desirable in younger individuals in whom the need for thyroid is more urgent and the difficulty in getting a true basal metabolic rate more apparent.

The analysis of the series of 100 test cases given here rests upon careful scrutiny of each individual case. In this respect a rather small number of cases has a distinct advantage over massed statistics in large numbers, such as have been reported in the literature. In particular, the consistency of the results here presented depends upon several factors, among which the following are especially important: 1, the utilization of plasma protein-bound iodine after excluding contact with unusual amounts of iodine, and 2, the segregation of cases of Graves' disease with hyperthyroidism from the ophthalmic cases otherwise known as "Graves' disease without hyperthyroidism." Because this segregation was made on clinical grounds, it is valid to conclude that the normal iodine values found in the plasma of these patients constitute additional evidence that Graves' disease and thyroid activity may be dissociated.[11] Among such patients are included former cases of chronic physiologic hyperthyroidism which are permanently or temporarily in remission, *i. e.*, the so-called "burned out" hyperthyroid patients.

In general, the clinical consistency of the data furnished by the 100 test cases and 10 euthyroid controls substantiates strongly the hypothesis raised in the previous communication[1] that plasma protein-bound iodine may be used to measure physiologic thyroid activity independently of the structural status of the thyroid gland.

Summary. A series of 100 cases of suspected thyroid disturbance and 10 control individuals has been analyzed from the standpoint of final clinical diagnosis, basal metabolic rate and plasma protein-bound iodine. When the first two of these criteria are in agreement, there is a close correlation between the plasma iodine and the basal metabolic rate. Such cases amount to 71% of the entire group studied. Of the remaining 29%, the basal metabolic rate often did not clearly reflect the clinical status and the plasma protein-bound iodine proved much more reliable. This was particularly

true in the group of "Graves' disease without hyperthyroidism" in which the basal metabolic rate ranged from −20 to +40%, but the plasma protein-bound iodine was normal. These data therefore constitute additional evidence for the possible dissociation of physiologic hyperthyroidism and clinical Graves' disease. In hypothyroidism, plasma protein-bound iodine appears to be a highly reliable criterion for confirming lack of thyroid hormone, even when full-blown myxedema is absent.

It has been emphasized that the simplified chemical procedures employed are partly empirical. Their use has been justified by their consistency with clinical data, but due care should be exercised in comparing the data with the results obtained by other methods.

CENSUS OF CASES STUDIED.

Case No.	Patient.	Age.	Sex.	Hospital No.*	Diagnosis.	B.M.R.	"P" iodine, μg.
1	T. R.	44	F	979256	Myxedema	−45	2.2
2	M. H.	28	F	M.G.H. 227433	Myxedema	−43	1.9
3	N. H.	41	F	972699	Myxedema	−41	2.7
4	H. W.	42	M	970183	Postoperative myxedema	−42	3.2
5	J. O'B.	16	F	966080	Hypothyroidism	−27	2.8
6	A. McL.	55	F	999743	Myxedema	−18	0.8
7	M. B.	73	F	M.G.H. 18190	Myxedema	−50	1.6
8	A. G.	56	F	M.G.H. 270469	Hypothyroidism	−23	2.4
9	C. L.	64	F	1001923	Hypothyroidism	−18	3.2
10	M. M.	56	F	1004489	Myxedema	−28	2.2
11	M. P.	62	F	M.G.H. 263055	Hypothyroidism	−21	1.6
12	H. D.	32	F	Private	Mild hypothyroidism	−13	3.9
13	O. P.	56	F	Private	Mild hypothyroidism	−14	3.3
14	G. M.	16	F	1015066	Hypothyroidism	−20	3.6
15	G. W.	54	M	Private	Myxedema	−43	1.3
16	M. D.	33	F	M.G.H.	Myxedema	−33	2.9
17	R. J.	47	F	M.G.H. 264633	Myxedema	−32	2.2
18	H. O'N.	42	F	1026368	Pituitary necrosis with myxedema	−44	1.6
19	L. C.	71	F	1028107	Myxedema	−19	2.7
20	E. M.	45	F	M.G.H. 236576	Hypothyroidism	−34	3.4
				**********			
21	G. R.	54	F	961449	Anxiety	+1	4.8
22	A. H.	46	M	Private	Tachycardia	+2	4.8
23	A. T.	29	F	1007421	Achondroplasia	+3	4.6
24	A. P.	17	F	Private	Simple goiter	−10	6.9
25	F. O.	44	F	Private	Hemangioma of skull	−1	7.4
				**********			
26	J. B.	27	M	M.G.H. 263917	Graves' disease	+37	14.9
27	M. D.	25	F	970024	Graves' disease	+66	10.1
28	N. E.	49	M	992137	Graves' disease	+56	11.5
29	J. H.	69	M	988024	Graves' disease	+27	10.5

* Unless otherwise designated, the hospital number refers to cases at the Boston City Hospital.

Case No.	Patient.	Age.	Sex.	Hospital No.*	Diagnosis.	B.M.R.	"P" iodine, μg.
30	M. K.	33	F	969633	Toxic nodular goiter recurrent	+60	11.3
31	E. L.	55	F	996982	Graves' disease	+40	10.9
32	C. M.	..	F	M.G.H. 258373	Graves' disease	+41	8.3
33	D. M.	16	F	M.G.H. 231166	Graves' disease	+54	13.1
34	F. M.	31	F	976942	Graves' disease	+50	11.4
35	P. W.	39	M	963562	Graves' disease	+28	14.6
36	B. H.	18	F	M.G.H. 231737	Graves' disease	+54	13.1
37	H. Y.	54	F	993938	Recurrent Graves' disease	+32	12.1
38	H. A.	20	F	M.G.H. 274523	Graves' disease	+52	10.8
39	D. D.	19	M	977308	Graves' disease	+49	11.8
40	J. S.	51	M	969996	Graves' disease	+70	14.9
41	J. W.	59	F	973628	? Toxic adenoma	+36	11.2
42	F. L.	62	F	Evans 272458	Toxic nodular goiter	+50	16.1
43	A. B.	28	F	1004547	Recurrent Graves' disease	+33	11.7
44	M. McL.	20	F	971348	Graves' disease with thyroid storm	†	18.2
45	T. C.	24	F	1006146	Graves' disease	+64	13.5
46	A. H.	15	F	1005920	Graves' disease	+33	13.4
47	D. L.	19	F	1023229	Graves' disease	+43	18.9
48	J. S.	56	M	1007278	Graves' disease	+50	16.0
49	W. McD.	52	M	1008987	Graves' disease	+47	9.5
50	A. C.	31	M	1008240	Graves' disease	+40	24.7
51	A. G.	59	F	1011753	Graves' disease	+18	9.4
52	F. W.	61	F	M.G.H. 284938	Graves' disease	+46	8.0
53	D. B.	51	M	M.G.H. 263273	Graves' disease	+41	9.2
54	A. N.	23	F	M.G.H. 285290	Graves' disease	+51	24.2
55	B. W.	19	F	M.G.H. 285466	Graves' disease	+33	13.2
56	G. C.	42	F	1023109	Graves' disease	+30	10.7
57	G. D.	40	F	998702	Graves' disease	+40	4.9
58	A. B.	47	F	1024937	Graves' disease	+70 (±)	19.0
59	J. J.	45	M	M.G.H. 301702	Graves' disease	+18	12.4
60	C. M.	51	F	1026912	Graves' disease	+26	9.0
61	J. E.	25	F	1028809	Graves' disease	+36	8.0
62	I. B.	59	F	Private	Toxic nodular goiter	+18	9.7
63	C. M.	48	F	Westfield State Hosp.	? Cancer of thyroid	+87	13.1
				**********			
64	L. C.	26	F	M.G.H. 272647	Unilateral exophthalmos	−4	6.1
65	F. L.	23	F	M.G.H. 276424	Bilateral exophthalmos	+11	7.0
66	B. H.	36	M	1017062	Bilateral exophthalmos	−10	6.4
67	M. G.	59	F	M.G.H. 280427	Bilateral exophthalmos	+8	6.7
68	A. M.	32	F	989448	Recurrent Graves' disease without hyperthyroidism	+8	6.4

* Unless otherwise designated, the hospital number refers to cases at the Boston City Hospital.

† Unobtainable.

Case No.	Patient.	Age.	Sex.	Hospital No.*	Diagnosis.	B.M.R.	"P" iodine. μg.
69	S. D.	17	F	968791	Recurrent Graves' disease without hyperthyroidism	+9	7.3
70	M. K.	31	F	995785	Graves' disease without hyperthyroidism	−3	4.2
71	A. R.	22	F	1028558	Graves' disease without hyperthyroidism	+2	5.3
				**********			
72	I. S.	28	F	971762	Tuberosclerosis; achondroplasia	−25	6.3
73	L. G.	43	F	976115	Intrasellar tumor	−20	5.2
74	M. S.	23	F	Private	Colloid goiter	−14	6.1
75	J. O'S.	36	M	1004320	Anxiety state	−22	5.7
76	B. M.	23	F	1002060	Hypoövarianism	−24	5.8
77	C. G.	19	F	M.G.H. 270785	Rheumatoid arthritis	−17	5.8
78	L. P.	23	F	Private	No complaint	−19	5.1
79	O. P.	28	M	Private	No complaint	−18	5.0
80	J. L.	51	M	Private	Hypopituitarism with hypogonadism	−30	4.3
				**********			
81	L. S.	36	F	Private	Anxiety state, colloid goiter	+22	4.6
82	E. C.	31	F	925968	Anxiety state	+21	5.2
83	M. N.	44	F	965392	Hypertensive and arteriosclerotic heart failure	+18	7.7
84	H. McL.	30	F	O.P.D. 613616	Anxiety, pregnancy	+29	6.6
85	C. McL.	35	F	995182	Cardiac decompensation; diffuse colloid goiter	+47	4.6
86	P. McK	52	M	967590	Hypertensive and arteriosclerotic heart disease, decompensated	+29	4.2
87	A. S.	25	F	999787	Anxiety state	+16 to +33	5.4
88	J. D.	37	M	965164	Lobar pneumonia (fever)	+42	4.0
89	M. C.	24	F	831983	Neurosis	+47	4.9
90	F. B.	59	F	M.G.H. 297531	? Parkinson's disease; post-thyroidectomy	+29 to +50	7.1
91	B. T.	68	F	1027816	Congestive failure	+60	6.9
92	L. A.	54	F	1028709	Congestive failure	+20	6.9
				**********			
93	B. B.	59	F	M.G.H. 275912	Bilateral exophthalmos	−18	4.5
94	M. F.	22	F	M.G.H. 271526	Unilateral exophthalmos	−12	4.0
95	M. C.	39	F	1028107	Bilateral exophthalmos	−20	5.3
96	H. H.	36	M	1017108	Post-thyroidectomy exophthalmos	−20	3.9
97	R. B.	22	M	1008456	Graves' disease without hyperthyroidism	−15	5.6
				**********			
98	E. K.	23	F	M.G.H. 199044	Exophthalmos; ? hyperthyroidism	+17	7.1
99	M. B.	41	F	977128 990460	Graves' disease without hyperthyroidism	+36	5.5

* Unless otherwise designated, the hospital number refers to cases at the Boston City Hospital.

Case No.	Patient.	Age.	Sex.	Hospital No.*	Diagnosis.	B.M.R.	"P" iodine, µg.
100	C. B.	58	F	O.P.D. 662563	Recurrent Graves' disease	+14	8.8
				**********			
101	S. S.	26	F	..	No disease	0	5.1
102	J. W.	26	M	..	No disease	−9	5.1
103	N. S.	27	F	..	No disease	−4	4.6
104	M. B.	29	M	..	No disease	−3	5.3
105	R. B.	28	M	..	No disease	+7	4.9
106	J. M.	27	M	982415	Acute bronchitis	−9	5.5
107	F. S.	52	M	977948	Peptic ulcer	−3	5.2
108	W. B.	19	M	974372	Empyema	−9	5.9
109	L. C.	69	M	964840	Hypertension	−3	5.7
110	M. G.	59	M	980912	Tabes dorsalis	+5	5.0

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EXPERIMENTAL ASPIRATION PNEUMONIA; FLUORESCENCE AND PATHOLOGY.†

By Irving J. Wolman, M.D.,

and

Anna B. Bayard, B.S.,

Philadelphia, Pa.

(From the Department of Pediatrics, School of Medicine, University of Pennsylvania, and the Children's Hospital of Philadelphia.)

The lung responds to the aspiration of foreign oils and fats by a diversity of related tissue reactions which generally are classified together, the condition being designated by a number of synonymous names, such as lipoid pneumonia, oil aspiration pneumonia, and, more recently, lipid pneumonia.[4] Although in routine autopsy

* Unless otherwise designated, the hospital number refers to cases at the Boston City Hospital.

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practice the origin of such lesions can often be recognized with the help of clinical information already at hand, identification of the offending substance in unknown specimens on morphologic appearances alone is difficult and often impossible.

Fluorescence of lipid pneumonia lesions in gross pathologic material has been described from this laboratory.[13] When affected human lungs are examined under ultra-violet light in a dark room the consolidations stand out as patches of pale yellow, gray, white or greenish fluorescence. That method of examination can show up small spots of involvement which escape detection under ordinary illumination.

This study was undertaken in order to investigate further the constancy of this fluorescent phenomenon, and the relationships, if any, between substances commonly aspirated and the color tones observed. Another purpose of this paper is to elucidate in more detail than at present understood, certain variable factors as they relate to the gross and microscopic structure of lung lesions. These factors include the different foreign substances most commonly inhaled by children: cod-liver oil, mineral oil, and milk; the pathologic changes which take place with time; the differences, if any, between reactions to single and multiple inhalations; and the behavior to special staining reagents. Inasmuch as lipoid-containing cells have been repeatedly described in milk aspiration pneumonias, a series of observations on aspiration pneumonia due to butter fat and milk free from fat have been included.

The literature on this condition is increasing rapidly, mainly because of the growing alertness of clinicians and radiologists to its occurrence. The most common offenders in humans are considered to be mineral oil, plain or medicated, cod-liver oil, castor oil, olive oil, and the butter fat of milk formulas. Their entry is usually by way of oily nose drops or by choking upon or swallowing improperly oily or fat-containing substances.

As recorded in the literature the gross appearance of affected human lungs is variable. The involved portions may be pale yellow, gray-white, or gray; large or small; sharply marginated or with indefinite borders; scattered or clumped. Accompanying bronchopneumonia usually obscures the specific consolidation. An oily appearance is given only by freshly aspirated material. The distribution of the lesions demonstrates the exogenous entry of the contained fat or oil, inasmuch as the posterior and inferior regions and the perihilar areas are most often affected, and the right lung usually is involved more extensively than the left.[3]

The microscopic reactions have also been described in great detail, and can be summarized as follows: The picture varies with the kind and amount of the aspirated lipids, the age of the lesions, and the character and extent of the complicating secondary pneumonia so frequently present. Oil globules may lie free in the alveolar

spaces or be encysted within mononuclear foreign body giant cells. Neutrophils are not present in any number except when the oil contains fatty acids or other irritant substances, or if bacterial invasion has taken place. Large macrophages take up and hold the oil droplets; at times these phagocytes coalesce to form giant cells of the foreign body type. The alveolar lining may take on a cuboidal or columnar shape when stimulated by medicament-containing mineral oil or by cod-liver oil. Following entry of irritating oils the immediate response is local hemorrhage and edema, fatal if the inflammation is sufficiently great. When bacteria in any abundance are present within the foreign material the inflammatory reactions become much more violent and can go on to abscess formation. The end-result of lipid aspiration is usually residual fibrosis with giant cell formation, round cell infiltration and granulomatous nodules. Much of the oil is disposed of through expectoration, some is destroyed *in situ*, some is carried off into the lymphatics, while the residue, of variable quantity, remains locally in granulomas within the lung.

A number of practical suggestions for use in identifying the various oils have been suggested, based on differences in the histologic architecture and staining responses of lesions in experimental and clinical material. Cod-liver oil, chaulmoogra oil, and lard are highly irritating, presumably because of their highly unsaturated fatty acids, whereas olive oil, sesame oil, and cottonseed oil are much more bland. Mineral oil, a hydrocarbon, is freely phagocytosed by macrophages and remains as a persistent inert foreign body encapsulated in fibrous granulomas, although when drugs such as ephedrine or camphor are contained in it the pulmonary reactions become more extensive.[8] Cod-liver oil gives rise to an acidophilic hyaline membrane which is acid-fast to carbol fuchsin as used in the staining of tubercle bacilli;[7,11b] this membrane persists for a long time, ultimately becoming phagocytosed. Confirming data can sometimes be obtained by chemical studies of the involved tissues.

Yet, in spite of the mass of observations on record, all details of the pathologic change have not been fully elucidated, especially with regard to the comparative importance of milk and milk products in the pathogenesis of the disorder.

Method. The foreign substances utilized in this study were as follows: 1, Cod-liver oil (U.S.P.); 2, mineral oil (U.S.P.); 3, canned evaporated milk diluted with equal parts of sterile distilled water; 4, fresh unsalted melted butter (not autoclaved), and 5, centrifuged autoclaved market milk containing less than 0.01% butter fat.

The experimental animals were young healthy New Zealand Red rabbits at sexual maturity. Though previously used in Friedman pregnancy tests, they had been given a 2- to 4-week recovery period and were free from snuffles and wound infections. Their weight ranged between 4 and 5½ lbs. at the onset of the observation period, and between 5 and 10 lbs. at the time

of killing. Five groups of such rabbits, 21 to 24 in each group, were taken. As controls, the lungs of some 20 uninoculated animals of comparable condition were studied in exactly similar fashion.

To simplify the comparisons all substances were instilled under essentially identical circumstances in accordance with the same fixed schedule. One to five instillations were given, spaced from 1 to 7 days apart. The oils were dropped into each nostril in 1 cc. doses; the milk preparations, warmed, in 5 cc. quantities.

The animals, lightly anesthetized with ether, were held vertically during the instillations. They were killed at progressive intervals of from 2 days to 1 year following the final treatment, by means of intravenous injections of air. Immediately afterwards the lungs were removed, and their gross appearances noted in ordinary light and under the fluorescent lamp. Ten per cent formalin solution was used as the hardening and preserving fluid. The ultra-violet ray observations were repeated several times later in order to determine the effects, if any, of this fixing fluid on the fluorescence phenomenon.

Paraffin sections made from grossly consolidated areas were stained with hematoxylin and eosin, and also with the acid carbol-fuchsin stain. Scarlet red staining of frozen sections was used for recognizing the fat globules. A fourth set of frozen sections have been mounted in water glass upon special ultra-violet transmitting glass slides and set aside for future study with the ultra-violet microscope.

The specimens were studied for the following points: gross appearances of the consolidated areas as seen by the naked eye and under the fluorescent lamp; histologic cellular reactions as related to offending foreign substances and to the intervals since the final inhalations; extent of the inflammatory response in comparison with the amount of foreign material demonstrable; length of time necessary for complete disappearance of the aspirated material; and subsequent presence or absence of residual alterations in the cellular architecture of the lung tissue.

Observations. *Distribution of the Lesions.*—The distribution of the foreign materials and of the pneumonic reactions to them proved to be more or less the same with all substances tested. The lesions were scattered unevenly through all parts of the lungs, usually being larger and more abundant in the lower lobes, presumably because of the upright position of the animals during the instillations. All parts of the lobes were involved, without noticeable selective localizations in the hilar regions. Subpleural lesions could be easily recognized on inspection. Wide fluctuations in size and distribution of the pneumonic patches were observed with each of the substances; some pairs of lungs being flooded with specific consolidations whereas others displayed only a few isolated spots. Clearly the quantities of instilled liquid which passed the laryngeal barrier were inconstant and unequal, in spite of every attempt to keep the experimental conditions constant.

Fluorescence. For the purpose of detecting fluorescence in lung specimens simple dark lamps such as geologists use were found suitable. With the shorter ultra-violet wave lengths (2400–2800 a.u.) the lung tissue emitted a dark red glow due to the hemoglobin, which persisted to obscure the colors of the pneumonic areas even after prolonged formalinization. But under the longer ultra-violet

waves (2800–3100 a.u.) the hemoglobin fluorescence of the background of lung tissue became practically invisible after a few days' contact with formaldehyde.

The fluorescence of the aspiration pneumonias usually grew more prominent when the tissues had been preserved for several months. This technique of repeated observation proved helpful in uncovering small patches of aspiration pneumonia not detectable under ordinary illumination.

Multiple Instillations. In the animals which had received multiple instillations over periods of several days the sections showed the changes of early acute inflammation phenomena side by side with more advanced subacute reactions.

Cod-liver Oil. The pneumonic patches looked yellow or yellow-brown in both fresh and formalinized specimens. Of all the experimentally produced aspiration lesions those of cod-liver oil were the largest, especially in their early stages when many possessed central foci of liquefaction. Under ultra-violet light the patches fluoresced brightly, showing color effects noted as yellow, pale yellow, cream, or yellow-brown.

Histologic changes bore a constant relation to the age of the lesions. In the youngest preparations (2 to 4 days) amorphous eosinophilic extracellular masses of oil surrounded by large numbers of phagocytes, neutrophils, eosinophils and monocytes were scattered through alveoli and bronchioles. Within the larger lesions central liquefaction soon occurred (4 to 7 days). Later on (7 to 10 days) the cells lining the alveoli sometimes swelled and became cuboidal; the neutrophils became less plentiful (Fig. 1). Most of the phagocytes were monocytes and eosinophils, with fibroblasts beginning to make an appearance. The amorphous deposits had become smaller and were being penetrated by the leukocytes. After 15 days, inflammatory granulation tissue encapsulated the oily débris and the abscesses had disappeared. Scar tissue and foreign body giant cells were replacing the earlier acute exudate. Even after 1 year these granulomas of giant cells, eosinophils, and fibrous tissue surrounding hyaline yellow-colored foreign material were still prominent (Fig. 2).

Scarlet red preparations showed an abundance of orange-red staining fatty material at all times. After the first few days the larger deposits were shown contained within syncytial cell masses, while the small drops were engulfed by individual lipophagic monocytes. Even after 2 months not much fine emulsification had taken place, though there was a definite trend toward reduction in droplet size with the advance of healing.

In the sections stained with carbol fuchsin the lipoid material appeared as acid-fast droplets, both intracellular and extracellular. The intensity of staining fluctuated somewhat among the various specimens, and was not striking in the more advanced lesions. In

the freshest lesions staining was deepest at the periphery of the oily masses.

Mineral Oil. In freshly removed lungs the affected areas appeared gray-white and bloodless, easily visible against the pink spongy

FIG. 1.

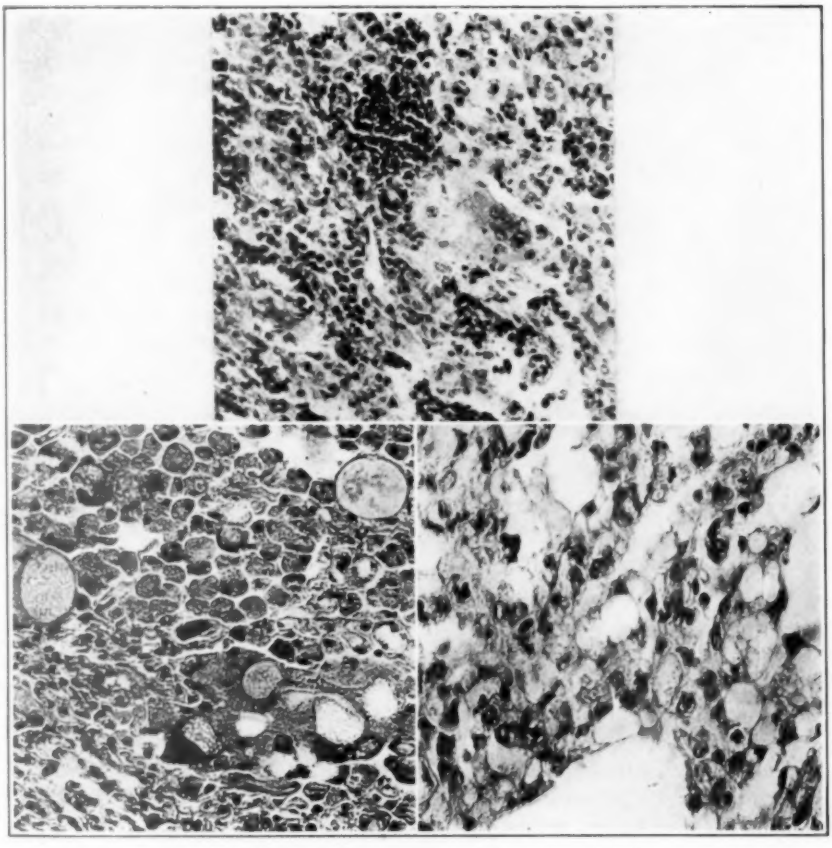

FIG. 2. FIG. 3.

FIG. 1.—Lung, 10 days after single inhalation of cod-liver oil. (× 400.) Lipid material is seen within a large multinucleated foreign body giant cell. A cluster of leukocytes, consisting chiefly of neutrophils, lies in close proximity. The neighboring mononuclears contain small droplets of oil.

The defects in these photomicrographs are in the original slides and not in the photographs or their reproduction.

FIG. 2.—Lung, 8 months after 3 inhalations at weekly intervals of cod-liver oil. (× 400.) Granular deposits of pale yellow lipid remain within large mononuclears and giant cells. Evidences of acute irritation are not present. An almost identical appearance was observed in the 12 months' specimen.

FIG. 3.—Lung, 15 days after single inhalation of mineral oil. (× 500.) Oil globules of varying sizes lie within the alveoli, either phagocytosed by single cells or surrounded by syncytial masses. No oil lies free in the alveolar spaces. The deeply staining small phagocytes are eosinophils. Neutrophils are rarely seen.

parenchyma of the surrounding lung tissue. After formalin fixation they were much less prominent, though always whiter than the pulmonary tissue background. Under the ultra-violet lamp they were manifest as pale gray spots tinted at times with an added faint green, green-blue, or blue color.

In the early stages (Fig. 3) the affected alveoli and terminal bronchioles were packed with a syncytial-like mass of phagocytes having cytoplasm filled with large and medium-sized vacuoles. In specimens but 2 days old many oil drops appeared to be large and extracellular, but after a few days they had become broken up, giving to the cross-section a foamy pattern. After some months, however (Fig. 4), a marked tendency to fine emulsification had become evident. With the passage of time the lining cells of the involved alveoli took on a cuboidal shape, their walls became thickened, and some vacuolated macrophages became deposited in the interstitial connective tissue. The only other tissue response was the appearance of eosinophils in small numbers in some specimens.

In 2 of the animals which had received multiple instillations patches of necrosis, hemorrhage and acute inflammatory reaction were discovered, but the presence of large colonies of bacteria in the Gram-stained preparation indicated the existence of a superimposed acute bacterial pneumonia.

With scarlet red the color assumed was orange yellow, much paler than that given by the cod-liver oil and butter fat preparations. Mineral oil did not stain with carbol fuchsin.

Evaporated Milk. Fresh lungs removed during the first 2 weeks following instillation of milk showed gray-white pneumonic mottling which remained paler than the surrounding tissue after fixation in formalin. Under ultra-violet light the pneumonia had a white color, better shown in the formalinized material. With ordinary illumination no consolidations were recognizable later than 2 weeks, although small fluorescent white spots persisted up to 30 days of age.

Within 2 days after inhalation the microscopic examination showed a pneumonic reaction so extensive that the contours of the alveolar network were completely obscured (Fig. 5). One saw a solid mass of fibrin, mononuclears, neutrophils, eosinophilic cells and fibrous tissue homogeneously commingled with some finely granular purple staining débris. Vacuolated monocytes were uncommon. After a few days the picture had not altered much except for diminution in the proportion of neutrophilic cells. Abscesses did not form. At one week the amorphous material could no longer be seen, and by 2 weeks the reactions had largely subsided, leaving small areas of scarred thick-walled alveoli, containing a few monocytes of irregular shape. At 1 month and later the lungs seemed completely recovered except for residual alveolar thickenings, apparently permanent, and a few eosinophilic cells seen here and there. At no time did the alveolar epithelium itself assume a cuboidal form.

In the scarlet red preparations, occasional monocytes filled with fine red globules were seen in small groups within the acute inflammatory exudate. These cells decreased in numbers during the

FIG. 4. FIG. 5.

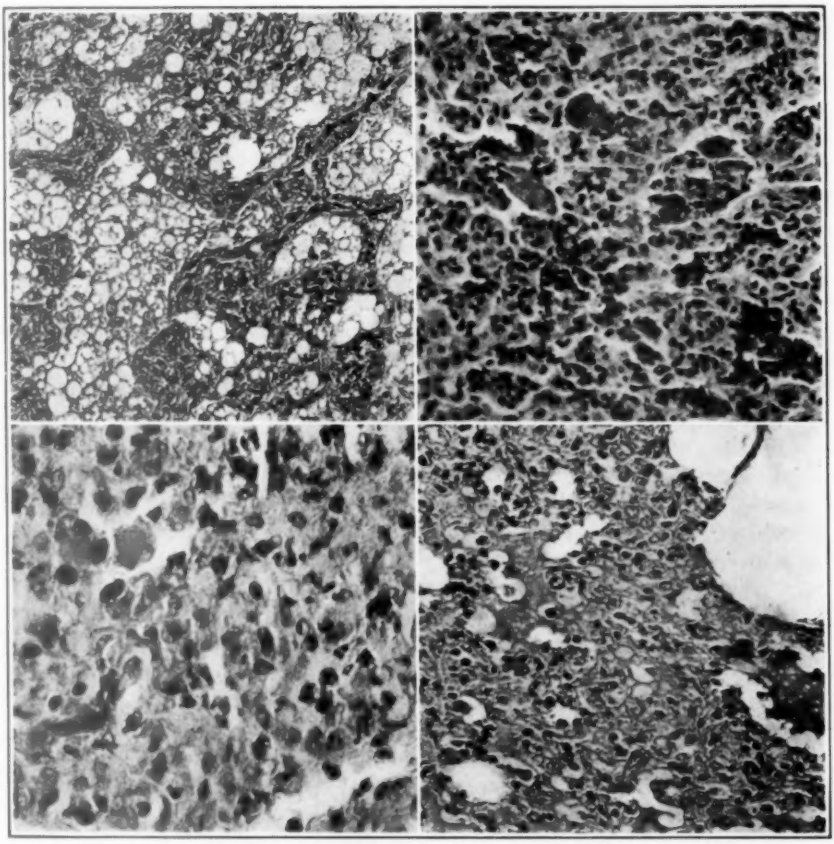

FIG. 6. FIG. 7.

FIG. 4.—Lung, 8 months after 3 inhalations at weekly intervals of mineral oil. (× 210.) Masses of vacuolated mononuclear cells containing large and small globules fill the alveoli. The alveolar walls are thickened and fibrosed. An almost identical appearance was observed in the 12 months' specimen. The deeply staining cells are eosinophils.

FIG. 5.—Lung, 2 days after single inhalation of evaporated milk. (× 400.) The alveoli are jammed with an intense inflammatory exudate consisting of neutrophils, edema fluid, basophilic coagulum, and mononuclear phagocytes.

FIG. 6.—Lung, 2 days after single inhalation of fat-free cow's milk, boiled. (× 566.) The exudate is less intense than with whole milk, neutrophils are less abundant, and basophilic débris is difficult to identify.

FIG. 7.—Lung, 2 days after single inhalation of melted butter. (× 300.) Butter, which stains deeply eosinophilic, is quickly liquefied. Note the mononuclear cells lying within lacunar pockets along the margins of the drops. The cellular response consists almost entirely of monocytes and eosinophils.

stage of resolution, and could not be found after 3 weeks. The carbol-fuchsin preparations were negative for the presence of oil residue.

Butter. In recently affected lungs the patches of consolidation were dark gray in color, more easily identified in the unpreserved specimen than after fixation. In the 1- and 2-month-old specimens no lesions could be seen. Under ultra-violet light the fluorescent effect was given by some but not all of the very early lesions, the color being faint gray-white tinged with green. In older inflammations the color was an indefinite white or gray and frequently could not be detected at all. Immersion in formalin intensified this white color; with some specimens recognition of the lesions in fresh tissue was difficult or impossible, but fluorescence studies subsequent to fixation demonstrated the involved areas.

On microscopic examination the immediate reaction to butter fat was found to consist of a patchy inflammatory exudate containing many monocytes, some neutrophils, and an occasional eosinophilic cell. The lipid material formed eosinophilic amorphous masses with scalloped margins (Fig. 6). Abscess formation did not occur and there were no acute inflammatory reactions which might be interpreted as a response to the bacteria present in butter. By the end of the week the picture was little altered except for the disappearance of the extracellular lipid. During the following week or two most of the reaction had faded away, but at 1 month and occasionally later one could find clusters of pigmented brown non-vacuolated irregular monocytes scattered among the alveoli.

With scarlet red, the butter stained vividly. After the first few days the butter fat had been completely phagocytosed and appeared as large intracellular globules. In time these globules multiplied and grew smaller, so that by 2 weeks they gave a stippled appearance to the cytoplasm. After 15 days prolonged search of the sections was needed to find a few cells having small intracellular fat globules; after 30 days none could be found. Carbol-fuchsin stains of the pneumonia area were negative for acid-fast matter.

Autoclaved Fat-free Milk. (This milk was sterilized and cooked by autoclaving for 30 minutes at 240 lbs. pressure and 115° C. temperature after being skimmed by high speed centrifugation. Fat content less than 0.01%.) The gross and fluorescent appearances of the pneumonia were identical with those of sterile evaporated milk. No fluorescent lesions could be found after 30 days.

In the paraffin sections the acute inflammatory reaction was similar to but not as violent as that observed with evaporated milk (Fig. 7). The exudate consisted largely of mononuclear cells. Débris and neutrophils were much less plentiful whereas the eosinophils were fairly numerous. By 10 days the neutrophils and débris had steadily receded and faded away and only foci of monocytic infiltration remained. At 1 month and later one saw only occasional thickened alveolar septa.

TABLE 1.—DIFFERENTIAL CHARACTERISTICS OF ASPIRATION PNEUMONIAS.

	Distribution of lesions.	Observed duration of lesions.	Color of gross lesions.	Central liquefaction at onset.	Ultra-violet fluorescence.		Intensity of acute inflammation in early stages.	Scarlet red stain.	Carbol fuchsin stain.	Fat particle size.	
					Recent aspiration.	Older lesions.				Recent aspiration.	Older lesions.
Cod-liver oil	All lobes, esp. in dependent regions	At least 1 yr.	Yellow or yellow-brown	++	Yellow	Pale yellow	++++	Orange-red	+	Large, irregular	Large and small
Mineral oil	All lobes, esp. in dependent regions	At least 1 yr.	Pale gray or white	0	White	Gray-white	++	Yellow	0	All sizes, rounded	Small
Evaporated milk*	All lobes, esp. in dependent regions	1 mo.	Gray-white	0	White	0	++++	Scarlet	0	Fine (rare)	0
Butter	All lobes, esp. in dependent regions	1 mo.	Dark gray	0	Gray-white with green tint	0	++	Scarlet	0	Large	Finely stippled
Autoclaved fat-free milk	All lobes, esp. in dependent regions	1 mo.	Gray-white	0	White	0	+++	0	0	0	0

* Water added.

No signs of fat infiltration were found in any of the frozen section preparations except for a few rare clusters of finely stippled macrophages in some of the animals which had been given multiple inhalations. The carbol-fuchsin preparations failed to reveal acid-fast matter.

Comment. The data presented show that under the conditions of the experiments the pathologic reactions produced by the different substances tested were individually characteristic (Table 1). Cod-liver oil provoked a violent necrotizing reaction which partially cleared to leave chronic granulomatous deposits. It was the only lipid in this series to possess acid-fast staining properties with carbol fuchsin. Mineral oil produced little acute inflammation and remained indefinitely as an unabsorbable foreign material. Butter fat gave rise to an immediate mononuclear response of transitory character. Evaporated milk stirred up a distinctive inflammatory reaction, also transient, in which the presence of small numbers of phagocytes holding fat could be considered as being entirely a response to its contained butter, inasmuch as no lipophages were found in the similar though milder experimental lesions produced with autoclaved fat-free milk. With the scarlet red stain butter fat appeared dark red, cod-liver oil was orange-red, and mineral oil orange-yellow, demonstrating decreasing rates of solubility in the order named. Under ultra-violet light the various pneumonias presented differing and individually typical color fluorescence. Further investigation has brought out that the fluorescent effects of these pneumonias are essentially the same as the colors given by the foreign materials themselves when examined in the pure state under ultra-violet light.

The effects of repeated inhalations were not strikingly dissimilar from those following single inhalations. Where the pathologic appearances changed quickly with progress of time the examinations showed lesions of various ages existing side by side. When the tempo of reactive change was more slow-paced it was impossible to distinguish between the lesions of single and multiple instillations.

The morphologic changes following milk aspiration, whether of normal fat content or fat-free, were of a character most unlike those of the oils and fats studied. There were no lipid deposits and the feature of chronicity was lacking. The pulmonary reactions were found due to irritation from the contained butter fat as well as from other components of the milk. If the designation "lipid pneumonia" persists in general use the milk aspiration lesions ought not to be included.

The observations reported here were restricted to rabbit lungs, but the findings are sufficiently clear-cut and distinctive to warrant the presumption that they parallel in general contour the pictures encountered in affected human necropsy material.

Conclusions. 1. The pathologic reactions in rabbit lungs to the introduction of cod-liver oil, mineral oil, butter, evaporated milk, and autoclaved fat-free milk have been studied.

2. Each of these foreign substances gave rise to characteristic changes sufficiently distinctive as regards gross appearances, histologic architecture, staining reactions, and fluorescent behavior to permit accurate identification, not only of the foreign substance itself, but often, also, of the age of the lesion.

3. The pathologist when studying autopsies should attempt to make specific diagnoses such as mineral oil pneumonia, cod-liver oil pneumonia, and milk aspiration pneumonia, rather than be satisfied with the less definitive terms aspiration pneumonia or lipid pneumonia.

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THE ACTION OF GLOBIN INSULIN COMPARED WITH THAT OF CRYSTALLINE, UNMODIFIED, AND PROTAMINE ZINC INSULIN.

By Garfield G. Duncan, M.D.,

CHIEF OF "B" MEDICAL SERVICE, PENNSYLVANIA HOSPITAL; ASSOCIATE PROFESSOR OF MEDICINE, JEFFERSON MEDICAL COLLEGE,

AND

Charles E. Barnes, M.D.,

RESIDENT IN MEDICINE, PENNSYLVANIA HOSPITAL, PHILADELPHIA, PA.

(From the Pennsylvania Hospital.)

An important aim in the treatment of diabetes mellitus with insulin is to give a sufficient quantity in one injection in conjunction with an adequate diet to maintain the level of the blood sugar within the limits of normal for a period of 24 hours. This is not possible, in the majority of patients, with any one of the insulins commercially available; namely, unmodified, zinc insulin crystals in solution (crystalline insulin) and protamine zinc insulin. The action of unmodified and crystalline insulin upon the blood sugar concentration occurs promptly and subsides within 6 to 10 hours after they are injected subcutaneously. Protamine zinc insulin is slow to act but its action persists from 24 to 36 hours after administration. Hence, in most cases, a single dose of any one of these three brands

of insulin causes a blood sugar lowering action which is either too rapid and too evanescent or too gradual for the satisfactory control of the diabetes. Satisfactory results are secured by combining these effects with the separate injection of two insulins, a rapidly acting and a slowly acting insulin.* This entails 2 or even 3 injections a day for the patient having severe diabetes.

To obtain satisfactory control of the diabetes by a single daily injection of insulin it would appear that for the majority of patients it would be essential to have an insulin which acts with sufficient rapidity to prevent hyperglycemia during the daytime, when three meals are taken in close succession, and with an action sufficiently prolonged to control the level of the blood sugar throughout the night. In this manner the shortcomings of the protamine zinc insulin and the crystalline insulin in the routine treatment of many patients who require insulin would be overcome.

There is evidence that globin insulin fulfills this purpose and affords certain other advantages. Bauman[2] in the first report on the clinical use of globin insulin found: 1, that its maximum hypoglycemic effect occurred about 8 hours after its injection, and that if too much was given before breakfast hypoglycemic reactions would occur in the late afternoon; 2, that a single dose of globin insulin controlled mild and moderately severe diabetes for a 24-hour period; 3, local allergic reactions at the site of injection were promptly relieved when globin insulin was substituted for protamine zinc insulin. Marks[5] has found that globin insulin "acts more strongly than protamine upon the breakfast carbohydrate and completes its action during the night with no perceptible carry-over." Andrews and his associates[1] found that fewer units of globin insulin were necessary to control hyperglycemia than when protamine zinc insulin was used. A single dose of globin insulin controlled severe cases of diabetes even when this end had not been accomplished with the combined use of crystalline and protamine zinc insulin. No nocturnal hypoglycemic reactions were observed when only one daily dose of globin insulin was employed. The maximum effect from globin insulin was manifested at approximately 8 hours after its injection and its effect became exhausted in about 15 hours.

The object of this investigation was to observe, under exactly controlled conditions, the effects of globin insulin as compared with the insulins now commercially available.

The globin fraction of globin insulin is prepared from beef blood, although it may be prepared from the blood of other animals, or from human blood. Globin is a simple protein which is soluble at its iso-electric point. It is obtained by removing the iron-containing chromogen fraction from the hemoglobin molecule. Globin does not yield histopeptone on hydrolysis, and therefore should not be

* Some authorities mix the rapidly and slowly acting insulins in the syringe and inject them simultaneously. This technique has been unsatisfactory in our hands.

classified as a histone.[4] The globin insulin used in this study was prepared in the Experimental Research Laboratories of Burroughs, Wellcome and Company. It contains 3.04 mg. of globin, 0.24 mg. of zinc, and 80 units of insulin per cc.[6] It is a clear, yellowish fluid having a pH of 3.7.

Methods.—Before attempting the routine treatment of diabetic patients with globin insulin it seemed advisable to determine the time of onset, the intensity, and the duration of the hypoglycemic effect produced by a single injection of this product and to compare the results with those produced by single doses of the types of insulin now in common use.

Forty-two patients from the medical wards were studied during this investigation; the results observed in 5 patients, each representative of a similar group, are reported in detail. They are: a non-diabetic adult (T.B.), an elderly man with very mild diabetes (A.Z.), a middle-aged man with mild diabetes (J.J.), a slender middle-aged man with severe diabetes (D.C.), and a young man of 18 years having very severe diabetes (H.M.). All of the patients were in the hospital for the duration of the study. During this period they were given weighed diets appropriate for their metabolic needs. Those who required insulin received their usual daily dose between the periods of observation. In order that the results might not be confused by the presence of insulin which was not yet absorbed and utilized, the subjects received no insulin for at least the 36 hours preceding the injection of the test doses. They received their usual meals during this period. The amount of insulin in the test dose given to each subject was determined on the basis of the severity of the diabetes as judged by his age, body weight, and the amount of insulin previously required. Upon the day of the test the insulin was given after venous blood had been obtained for determination of the fasting blood sugar level.* To maintain a uniform intake of carbohydrate throughout the period of observation, fruit juice, containing 20 gm. of carbohydrate, was given to each subject at the time of the administration of the insulin and at intervals of 2 hours thereafter until the conclusion of the test. Determinations of the blood sugar level were also made at intervals of 2 hours. In almost all instances the observations were continued until the blood sugar values had risen from the hypoglycemic values to the original fasting levels. The observations were repeated at intervals of at least 1 week until all three insulins were tested.

Case Reports. Case 1.—T.B., a white male, aged 47, height 172.7 cm. (68 inches), weight 70.8 kg. (156 pounds), was admitted on December 22, 1939, because of vomiting and a history of "tarry stools." Studies failed to reveal any pathologic change in the gastro-intestinal tract. There was no evidence of diabetes mellitus. He was given a diet containing protein 70 gm., carbohydrate 175 gm., and fat 83 gm. (1750 calories). No insulin was given except upon the days selected for the tests. The dose of insulin selected for this patient was 15 units, as this was considered sufficient to give a moderate degree of hypoglycemia without producing unconsciousness. The fasting blood sugar concentration varied between 84 and 100 mg. per 100 cc. Following the separate administration of unmodified and of crystalline insulin there was in each instance a reduction in the blood sugar level within 4 hours to a level of 76 and 49 mg. per 100 cc., respectively. The fasting level was restored within 10 hours after the injection in both instances. When protamine zinc insulin was given, the blood sugar level fell very slowly from a fasting level of 97 mg., reaching 77 mg. per 100 cc. in 10 hours and maintaining a low level for 21 hours. When globin insulin,

* Determinations of the blood sugar level were made using Benedict's modification of the micro-method of Folin and Wu.

15 units, was given, the blood sugar level fell from a fasting value of 100 mg. to 78 mg. per 100 cc. in 4 hours. The blood sugar concentration remained fairly constant about this level until 20 hours had elapsed. The fasting level was restored by the end of 22 hours (see Chart 1).

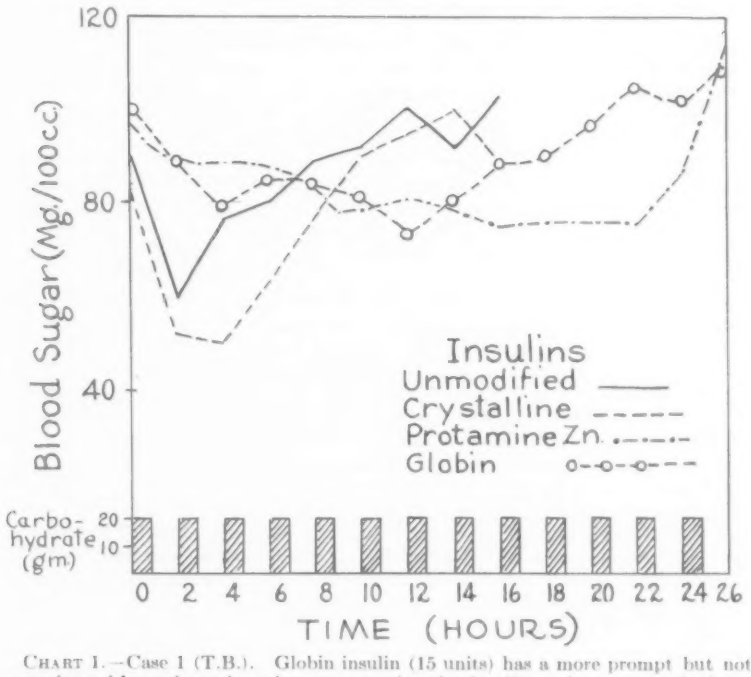

CHART 1.—Case 1 (T.B.). Globin insulin (15 units) has a more prompt but not as prolonged hypoglycemic action as protamine zinc insulin, and a more gradual and more prolonged action than the same doses of crystalline and unmodified insulins.

Comment. There is a contrast in this non-diabetic subject between the effects of 15 units of unmodified and of crystalline insulin, both of which had a rapid but evanescent action, and protamine zinc insulin, which had a retarded and prolonged action. The action of globin insulin like that of unmodified and crystalline insulin began soon after its injection but was continued for a much longer time than was the case with either of these insulins. This prolongation of action is, however, slightly less than that observed with protamine zinc insulin.

CASE 2.—A.Z., a white male, aged 62, height 165.7 cm. (66 inches), weight 61.2 kg. (135 pounds), was admitted on September 8, 1939. He had a history of diabetes mellitus of 9 years' duration. This patient had never required insulin and he was free from glycosuria when the diet contained protein 80 gm., fat 68 gm., and carbohydrate 140 gm. (1500 calories). For the purposes of this study 30 units of each of the insulin preparations were employed. Unmodified insulin reduced the blood sugar level from 90 to 51 mg. per 100 cc. during the first 4 hours. The blood sugar returned to the fasting level by the 14th hour. A similar, but more rapid reduction in the

blood sugar concentration was observed after the administration of crystalline insulin. Protamine zinc insulin reduced the blood sugar level, particularly after 4 hours, to reach a minimum of 53 mg. per 100 cc. in 18 hours. The fasting value was not regained in 28 hours. When globin insulin was given, the blood sugar values rose during the first 4 hours from 91 to 114 mg. per 100 cc., after which a prompt reduction ensued. The fasting level was not regained until the 32nd hour (see Chart 2).

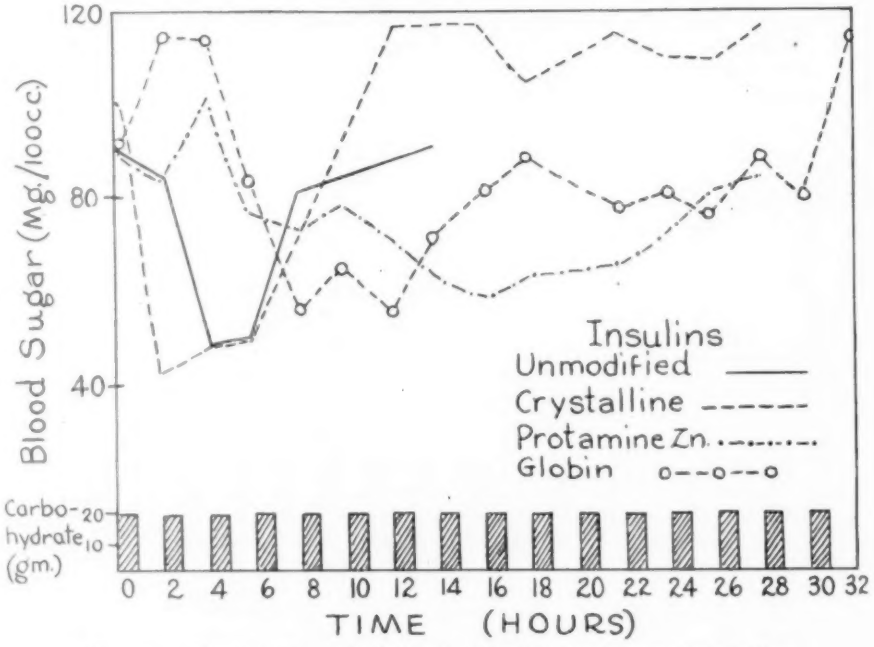

CHART 2.—Case 2 (A.Z.). Globin insulin (30 units) has the same intermediate effect as in Chart 1.

Comment. Unmodified and crystalline insulin each caused a prompt but fleeting reduction of the blood sugar level. Protamine zinc insulin exerted a gradual but prolonged blood sugar lowering effect. The effect of globin insulin fell between that of the rapidly and slowly acting insulins. It reduced the blood sugar level more quickly than did the protamine zinc insulin and more slowly than did the crystalline insulin. Its effect persisted longer than that of crystalline insulin and yet not as long as that of the protamine zinc insulin.

CASE 3.—J.J., a colored male, aged 56, height 173.9 cm. (68½ inches), weight 76.2 kg. (168 pounds), was admitted on January 9, 1940. His diabetes was of 2 years' duration. On a previous admission he received a diet containing protein 72 gm., fat 132 gm., and carbohydrate 132 gm. (2000 calories) and 48 units of protamine zinc insulin, and 18 units of unmodified insulin before breakfast each morning. During this investigation the same diet was prescribed. On successive weeks he was given a

test dose of 48 units of globin, protamine zinc and crystalline insulin respectively. Globin insulin promptly reduced the blood sugar concentration from 154 mg. to 50 mg. per 100 cc. during the first 6 hours following which the blood sugar concentration rose gradually to exceed the original fasting level at the 24th hour (see Chart 3). After the administration of protamine zinc insulin the blood sugar level rose slightly from 140 to 145 mg., in the first 2 hours and then fell gradually to levels between 97 and 137 mg. per 100 cc. which were maintained until the conclusion of the period of observation 28 hours after administration. Prior to giving 48 units of crystalline insulin, the fasting value of the blood sugar was 121 mg. per 100 cc.; the level rapidly fell to 35 mg. in the first 2 hours after the insulin was given and then it gradually rose to levels above the fasting value at the 22nd hour.

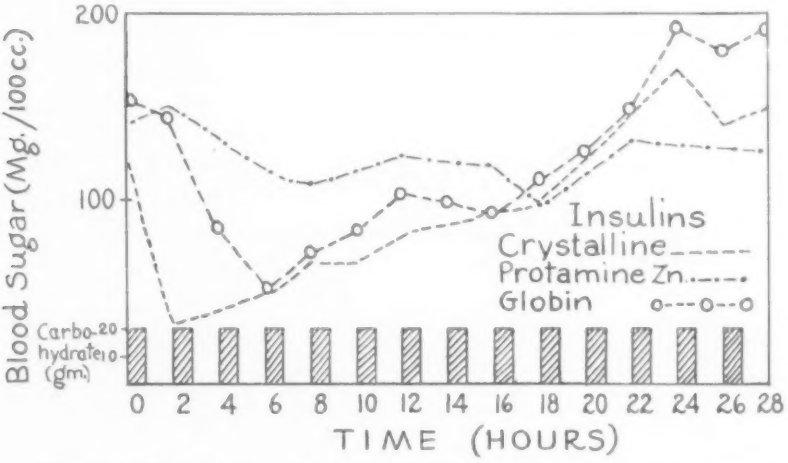

CHART 3.—Case 3 (J.J.). The intermediate effects of 48 units of globin insulin are depicted. The mildness of the diabetes prevents making conclusions concerning the duration of the effects of the respective insulins.

Comment. The immediate hypoglycemic response following the injection of crystalline insulin is in contrast with the slightly slower response to globin insulin and the very gradual lowering of the blood sugar level after protamine zinc insulin was given. The similarity of the curves obtained from plotting the blood sugar values obtained between the 6th and 24th hours following the injection of crystalline and globin insulin is a phenomenon often observed in patients who have a mild diabetes in whom the onset of insulin action is evident but in whom it may be impossible to tell when it ends. In these patients the blood sugar level may not rise promptly when the insulin action is exhausted.

CASE 4.—D.C., a white male, aged 55, height 162.5 cm. (64 inches), weight 61.2 kg. (135 pounds), was admitted on August 28, 1939, with a history of having had diabetes approximately 2 years. A diet containing protein 70 gm., fat 116 gm., and carbohydrate 200 gm. (2200 calories) was prescribed. The test dose of 78 units of unmodified insulin reduced the blood sugar level from a fasting value of 180 mg. to 41 mg. per 100 cc.

during the first 4 hours after which it rose, reaching 216 mg. 12 hours after the injection. After 78 units of protamine zinc insulin were given there was a rise in the blood sugar level from 183 mg. to 209 mg. per 100 cc. during the first 4 hours and then a reduction ensued and values within the limits of normal were maintained from the 10th to the 26th hour. The same amount of globin insulin (78 units) caused the blood sugar level to fall from a fasting level of 214 mg. to 58 mg. during the first 2 hours, to 38 mg. per 100 cc. in the next 2 hours. Subsequently, the values increased slowly until the 18th hour, after which it quickly rose to the fasting level (see Chart 4).

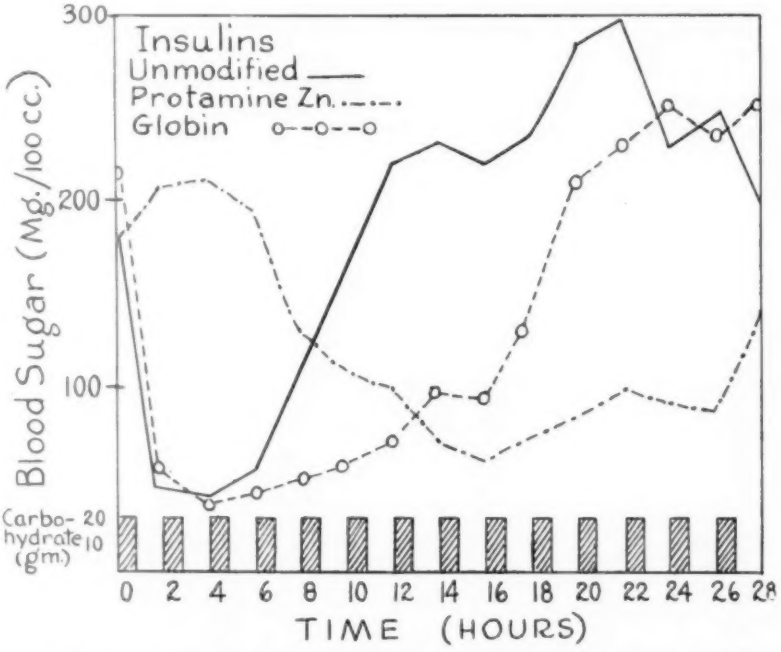

CHART 4.—Case 4 (D.C.). The speed with which the blood sugar levels were reduced and the duration of the hypoglycemic effects of 78 units each of globin, crystalline and protamine zinc insulins are demonstrated.

Comment. In this patient, with a more severe diabetes than had the preceding patients, the contrast between the effects of the various types of insulin is even more clearly discernible. The time of the onset of insulin action is clear-cut and the blood sugar concentrations rose promptly when the insulin action was exhausted. The effect of unmodified insulin was at an end by the 10th hour, that of protamine zinc insulin was exhausted at the 28th hour, and that of globin insulin at the 20th hour.

CASE 5.—H.M., a white male, aged 18, height 165 cm. (65 inches), weight 56.7 kg. (125 pounds), was admitted on January 5, 1940, with a history of having had diabetes one year. He was allowed a diet containing

protein 100 gm., fat 133 gm., and carbohydrate 300 gm. (2200 calories), and he was found to require 75 units of protamine zinc insulin with 32 units of unmodified insulin in the morning and 18 units of unmodified insulin before supper. Following the test dose of 80 units of unmodified insulin there was a prompt reduction of the blood sugar level from 264 mg. to 58 mg. per 100 cc. during the first 4 hours. After the 10th hour the blood sugar rose rapidly to high levels. A similar effect was observed following the injection of 80 units of crystalline insulin. After 80 units of protamine zinc insulin were given the blood sugar rose from a fasting level of 247 mg. to 284 mg. per 100 cc. during the first 4 hours after injection, and then gradually fell, reaching normal values between the 14th and 18th hours. The level then rose slowly although the fasting level was not regained by the 34th hour after injection of the insulin. When 80 units of globin insulin were given the blood sugar values fell, at first slowly, and then more rapidly, reaching a level of 84 mg. per 100 cc. at 10 hours after the insulin was given. The blood sugar level rose from 95 to 173 mg. per 100 cc. between the 24th and 26th hours (see Chart 5).

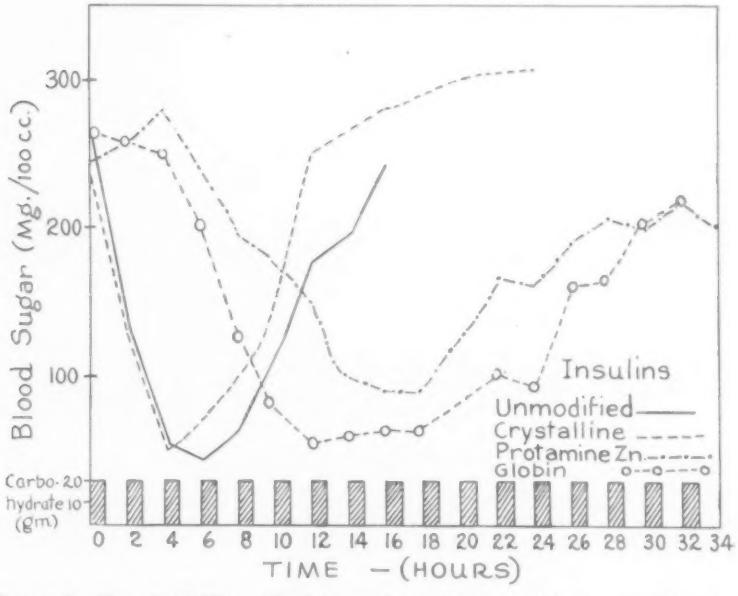

CHART 5.—Case 5 (H.M.). The intermediate effect of globin insulin (80 units) in a patient having a severe diabetes is depicted.

Comment. The results in this case depict: 1, The similarity of the speed, intensity and duration of the effects of unmodified and of crystalline insulin as has been previously observed and reported.[3] 2, The definite delay in the effect of the protamine zinc insulin is typical of the results which have been described by many observers. 3, The effect of globin insulin on the blood sugar level was apparent within 2 hours and was most marked between the 4th and 12th hours, maintaining a normal or hypoglycemic blood sugar level until the 24th hour. When a larger dose (120 units) was given, in a

TABLE 1.—BLOOD SUGAR VALUES DEPICTING THE COMPARATIVE HYPOGLYCEMIC EFFECTS OF IDENTICAL QUANTITIES OF THE VARIOUS SPECIFIED INSULINS.

Case No.	Pt.	Sex.	Age.	Ht. (cm.).	Wt. (kg.).	Type of insulin.	Dosage (units).	Blood sugar (mg./100 cc.) shown in hours after dosage.																	
								Fasting.	2.	4.	6.	8.	10.	12.	14.	16.	18.	20.	22.	24.	26.	28.	30.	32.	34.
1	T.B.	M	47	172.7	70.8	Unmodified	15	92	60	76	80	89	92	101	92	105									
						Crystalline	15	84	51	49	65	77	90	96	101	89									
						Protamine zinc	15	97	89	89	88	..	77	81	78	74	76	..	76	87	114				
						Globin	15	100	89	78	86	83	81	74	80	89	90	98	104	102	107				
2	A.Z.	M	62	165.7	61.2	Unmodified	30	90	84	51	52	81	..	..	93										
						Crystalline	30	100	45	51	51	71	99	117	..	117	104	..	116	107	107	118			
						Protamine zinc	30	90	81	102	77	73	77	..	63	59	53	..	67	..	80	85			
						Globin	30	91	114	114	83	56	66	56	72	82	90	..	77	80	75	88	80	114	
3	J.J.	M	56	173.9	76.2	Crystalline	48	121	35	44	47	69	70	83	89	96	97	..	148	167	140	146			
						Protamine zinc	48	140	145	137	128	110	..	122	120	120	97	..	132	130	129	129			
						Globin	48	154	143	87	50	74	85	103	98	92	110	127	147	193	180	189			
4	D.C.	M	53	162.0	61.2	Unmodified	78	180	47	41	56	117	172	216	233	217	239	283	298	235	246	198			
						Protamine zinc	78	183	205	209	197	132	109	95	68	60	68	85	98	90	86	137			
						Globin	78	214	58	38	41	48	57	71	96	94	131	210	229	249	234	249			
5	H M.	M	18	165.0	56.7	Unmodified	80	264	130	58	53	65	118	178	193	243									
						Crystalline	80	236	125	53	67	97	145	246	..	280	286	..	303	305					
						Protamine zinc	80	247	258	284	238	195	183	152	100	93	91	..	167	161	189	205	199	226	207
						Globin	80	264	259	253	205	127	84	55	58	63	64	..	106	95	173	176	203	223	
						Globin	120	246	270	215	145	78	56	58	46	39	48	..	60	60	64	77			

subsequent test, the blood sugar fell more rapidly and the lower level was maintained for a longer period, illustrating the importance of the size of the dose in considering the duration of action of any type of insulin.

Discussion. Globin insulin, in all but one of the subjects upon whom observations were made, had a blood sugar-lowering action which began within 2 hours after its injection. This effect reached a maximum intensity between the 2d and 8th hours, and was maintained for 18 to 24 hours. The hypoglycemic action during the first 4 hours was slower than that of identical doses of unmodified and crystalline insulin but more rapid than that of protamine zinc insulin. The period of maximum effect was observed to begin sooner and ceased somewhat earlier than when protamine zinc insulin was given. Thus from a practical application the quicker action of globin insulin tending to prevent postcibal hyperglycemia would reduce the need of supplemental doses of unmodified or crystalline insulin.

The blood sugar level reached the lowest point from the 8th to the 10th hour after the administration of globin insulin. This corresponds with the observations of Bauman that patients receiving globin insulin, in their routine treatment, are prone to have hypoglycemic reactions between 4 o'clock in the afternoon and dinner time unless appropriate shifting of the diet is made to prevent them.

In the patients who received moderately large doses of insulin (Cases 3, 4 and 5), there was a slightly greater hypoglycemic effect from the globin than from the protamine zinc insulin. Our attention was first drawn to this phenomenon during the observations upon patient H.M. (Case 5). This patient's total daily requirement had been 125 units (protamine zinc and crystalline insulin). We, therefore, decided upon 120 units as a proper test dose of each insulin. When this amount of globin insulin was given, his blood sugar level reached 56 mg. per 100 cc. on the 10th hour and normal values were not restored until the conclusion of our observations at the 28th hour. Subjectively, he was uncomfortable throughout this period and was semi-stuporous part of the time. The observations were repeated using 80 units as the test dose. Even with this amount of globin insulin the blood sugar level was as low as 55 mg. as compared with a minimum value of 91 mg. when an equal quantity of protamine zinc insulin was administered. This observation confirms our clinical experience in the routine treatment of diabetic patients; namely, that unit for unit globin insulin has a greater hypoglycemic effect than protamine zinc insulin. These studies will be the subject matter of another publication.

Summary. Observations on the comparative effects of equal quantities of unmodified, crystalline, protamine zinc and globin insulins have been made on 42 patients and the results reported in

4 diabetics and 1 normal control, all of whose carbohydrate intakes were uniform throughout the tests.

In each instance there was a distinct contrast between the effect of either unmodified or crystalline insulin which begins quickly and is soon ended, the effect of the protamine zinc insulin which is delayed in appearing and is prolonged, and the effect of globin insulin which fell between these extremes.

The blood sugar-lowering effect of globin insulin began soon after injection of the insulin and gradually increased in intensity until between the 8th and 12th hours after the injection. The effect then gradually subsided. The apparent effect of globin insulin in the non-diabetic and 2 subjects having a mild and 1 having a severe diabetes persisted for 20 hours after it was injected. In 1 patient having severe diabetes and receiving a larger dose the effect persisted for 24 hours.

Globin insulin has a quantitatively greater effect, unit for unit, than protamine zinc insulin.

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STUDIES ON THE HEMORRHAGIC AGENT 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN).

I. Its Effect on the Prothrombin and Coagulation Time of the Blood of Dogs and Humans.*

By James B. Bingham, M.D.,
INSTRUCTOR OF MEDICINE,

Ovid O. Meyer, M.D.,
ASSOCIATE PROFESSOR OF MEDICINE,

AND

Frederick J. Pohle, M.D.,
ASSISTANT PROFESSOR OF MEDICINE,
MADISON, WIS.

(From the Department of Medicine, University of Wisconsin Medical School.

In 1921 Schofield[18a,b] in Canada described a serious and sometimes fatal hemorrhagic disease in cattle which he attributed to the ingestion of spoiled sweet clover hay or silage. At about the same time Roderick[16a,b,17] in this country studied the condition

* This study was aided in part by a grant from the Wisconsin Alumni Research Foundation.

extensively and concluded that the cause of the delayed coagulability was a gradual diminution in the prothrombin of the blood. Quick[14] confirmed this conclusion. In 1934, Prof. K. P. Link and his associates of the Wisconsin Agricultural Experiment Station at the University of Wisconsin undertook the task of identifying the toxic factor in spoiled sweet clover. This work established the fact that the hemorrhagic agent was a dicoumarin compound. Later they succeeded in isolating and crystallizing the active principle, and have synthesized the biologically active substance and a large number of active pure crystalline derivatives. This splendid work was fully described in a series of publications[4–7,11,12,21] in the fall of 1940 and spring of 1941. In pure state this hemorrhagic agent is optically inactive, has a low solubility in ordinary organic solvents but is soluble in dilute alkalies. It was unequivocally demonstrated by Stahmann, Huebner and Link[21] through degradation reactions and by synthesis that the hemorrhagic agent, $C_{16}H_{12}O_6$, was the dicoumarin 3,3′-methylenebis (4-hydroxycoumarin). The chemical, physical and biologic properties of this product and of the agent occurring naturally in spoiled sweet clover were found to be identical.

In the early studies of hemorrhagic disease of cattle, Roderick observed no significant changes in the non-protein nitrogen, blood calcium, acid-base equilibrium, or icterus index. Occasionally the blood sugar was high, usually it was normal. Schofield[18b] found upon postmortem examination gross lobular lesions in the liver to which he attributed the hemorrhagic tendency. Roderick,[16a,b] on the other hand, observed only areas of focal necrosis, and these in but half of the cases. He believed that the lesions described by Schofield were due to postmortem change. Roderick found little evidence of serious impairment of the liver function. The hemorrhages occurred at different sites but were especially frequent in the subcutaneous tissues and in the fascia of muscles. Gross and microscopic examination of the blood-vessels did not show changes that would explain the hemorrhage.

It was recognized that if the disease with hemorrhagic manifestations was not too far advanced, it could be cured by eliminating the spoiled clover from the diet or by injecting blood serum or whole blood from healthy animals.

Experiments performed on rabbits by Quick[14] indicated that the feeding of alfalfa would counteract the bleeding tendency resulting from the feeding of toxic clover, but Smith[19] reported that alfalfa failed to prevent hemorrhagic sweet-clover disease. Some observations to be reported here tend to confirm the substance of these latter investigations.

We had followed with much interest the studies of Professor Link and his associates, for it was obvious that this substance might have important clinical application as a substitute for heparin in the

prevention of thrombosis. The considerable difficulty of purifying heparin, the resultant high cost, and the necessity for repeated or continuous intravenous administration made it desirable that similar agents without these handicaps be sought. The relatively low cost and the efficacy with which 3,3′-methylenebis (4-hydroxycoumarin) can be orally administered made the possibility of utilizing this material attractive.

With the synthesis of the hemorrhagic substance, large, relatively inexpensive quantities were made available for study upon larger animals and, if found feasible, upon human beings. This material was first furnished us through the kindness of Professor Link in September of 1940, since which time studies upon the pharmacologic and clinical properties have been made. The very generous cooperation of Dr. Mark Stahmann and Professor Link has facilitated this work.

It is the purpose of this communication to relate our experiences with this synthetic dicoumarin.

Prior to our study of this dicoumarin it had been established that this material produced prolongation of prothrombin time and delay in the coagulation of blood in rabbits and in dogs. It appeared to us that subsequent investigations should be directed, first, to elucidate the morphologic changes that might occur in dogs when so-called therapeutic and toxic doses were given; second, to establish the range between the effective therapeutic dose and the minimal lethal dose and thus determine the margin of safety; third, to determine whether this material reduced prothrombin in human beings as it does in cattle, rabbits and dogs; and fourth, to demonstrate, if possible, whether the administration of this dicoumarin in safe dosage would actually prolong the time of or prevent intravascular clotting. The fifth and very important point was, of course, to establish whether or not the material would prevent the development of thromboses in human beings; everything preceding is directed toward this major aim which can be settled only by extensive investigations in the future. Finally, the mode of action of the dicoumarin is important, but this matter has been little investigated to date.

Methods. Prothrombin time was determined on undiluted plasma by Pohle and Stewart's[13] modification of Quick's technique. In both dogs and patients, one or more normal controls were always utilized for check upon the technique and potency of the thromboplastin. Thromboplastin giving a normal time of 10 to 11 seconds in human beings and 5.5 to 6.5 seconds in dogs was used. For convenience, the approximate percentages of prothrombin in human plasma were derived from the chart of Pohle and Stewart. By this estimation, a time of 12.5 seconds indicates concentration of about 50% prothrombin; 19 seconds, 25% prothrombin; and 30 seconds, 12.5% prothrombin. In dogs a prothrombin time of 7 to 8 seconds indicates an approximate reduction of prothrombin to 50% of normal; 9.5 to 11 seconds to 25% of normal; and 15 to 20 seconds to about 12.5% of normal. A time longer than 45 seconds was generally less accurate because of the

greater difficulty in determining the end point. The work of Link[12] and his associates indicates that the concentration of the plasma used will affect the absolute values (% prothrombin). The values of prothrombin in per cent are therefore presented with reserve.

The coagulation time was measured by the 2-tube method of Lee and White at room temperature. The tubes were scrupulously clean and the syringe clean and wet. Care was taken to avoid too great suction in the collection of samples, to avoid air bubbles, and to transfer the blood to the test tubes with a minimum of agitation. Times longer than 14 minutes in humans and 12 minutes in dogs were felt to be abnormal.

Materials. 3,3′-methylenebis (4-hydroxycoumarin) was used exclusively in oral administration experiments and the disodium salt of this substance was the only material used intravenously. These materials were furnished to us ready to use by Stahmann and Link.

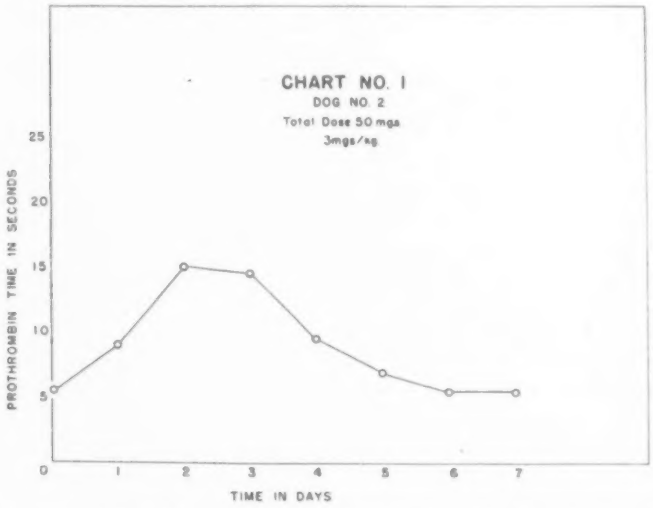

CHART 1.—The effect of a single oral dose of 3,3′-methylenebis (4-hydroxycoumarin) upon the prothrombin time of a dog.

Experiments. In all early experiments the powdered substance was given by mouth in gelatin capsules, in varying doses, to dogs that had not been fasted. In later experiments, when the approximate effective and safe dosage had been established, the disodium salt of 3,3′-methylenebis (4-hydroxycoumarin) was given intravenously to exclude the factor of variable absorption. A fairly characteristic effect upon prothrombin time of a single dose of the dicoumarin is shown in Chart 1 where 50 mg. (3 mg. per kilo) was given to Dog 2. Twenty-four hours after the material was administered the prothrombin time was slightly longer, still longer after 48 hours, attained its maximum after 48 hours, and then gradually returned to approximately the normal level on the 6th day. Chart 2 shows the effect of administration of repeated large doses to a dog

(No. 73) in which marked changes in both the prothrombin time and coagulation time were effected.

Observations in Dogs. Link and his associates demonstrated that the 3,3′-methylenebis (4-hydroxycoumarin) was capable of producing deficiency of prothrombin in the blood of animals. Heretofore, it had been demonstrated that prothrombin deficiency in man is caused by lack of absorption of the fat-soluble vitamin, as in obstructive jaundice, enteric disease, idiopathic steatorrhea, sprue, and gastro-colic fistulæ.[1,3,9,20] Deficiency of vitamin K in the newborn is presumably due to failure of bacterial synthesis.[15] Prothrombin

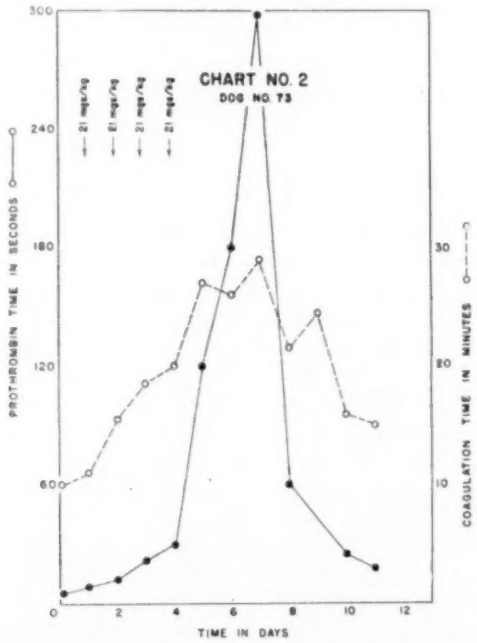

CHART 2.—The effect of repeated doses of 3,3′-methylenebis (4-hydroxycoumarin) upon the prothrombin time and coagulation time of a 14-kg. dog.

deficiency is also associated with extensive hepatic disease and here the administration of large quantities of vitamin K is often not corrective because the liver is unable to synthesize prothrombin.[8,13] Hence, despite the report of Roderick that no gross lesion was found in the liver of cattle dying of hemorrhagic sweet clover disease, it was our feeling that one might be dealing with a potentially dangerous substance. Therefore, the early studies were directed toward evaluating the toxicity of this material in dogs.

Originally the material was given in large and repeated doses, as shown in Table 1.

TABLE 1.—EFFECT OF REPEATED ORAL DOSES OF 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN) ON PROTHROMBIN AND COAGULATION TIME IN DOGS.

Dog No.	Dog wt., kg.	Days: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Remarks.
2A	16.7	6*	10	38	16	300	150	17	7.5		38		300					36	20?	80	2 hrs. (+)	24 hrs. (+)		4 hrs. (+)	Dog died 22d day; hemorrhages.
		5†	*5*	*6*	*7*	*10*	*7*	*10.5*	*3.5*		*4.5*		*7*			*11*		*4*	*13*	*7*	*6.5*	*10*		*11*	
		90‡								30		12				60		30		30				30	
3A	12.9	6.5		23				15		20	6 hrs. (+)	21 hrs. (+)													Found dead 13th day; G. I. hemorrhage.
		3.5		*4*						*5*	*6*	*18.5*	*27*	*18*											
		30						46		23															
5	15.8	8.5	38					360	1800 (+)	350	2 hrs. (+)	24 hrs. (+)													Died on 11th day; bleeding gums; blood in stool.
		3	*5*			*5*		*23*	*50*	*26*	*31*	*31*													
		32				64		32																	
6	17.7	7		40	28	58	2 hrs.	24 hrs.			4 hrs.	54	13												Hemorrhage from G. I. tract on 9th, 10th, 11th days; recovered.
		5			*21.5*	*10*	*10*	*16*	*5*		*3¼*	*15*	*7*												
		57		29		29			29																
7	16.2	7		65	44	95																			Died on 9th day; tarry stools; subcutaneous hemorrhages.
		7.5		*1¼*	*1¼*	*18*																			
		30		30		12																			
11	19.7	5.5	9	14		17	35			49				6.5 hrs.											Killed on 12th day.
		5	*5*	*5*						*7.5*				*30*											
		2.5		2.5		2.5		2.5					2.5												
12	13.5	6.5	10	22																					Died on 3d day; no hemorrhages, but widespread small vessel dilatation.
		¼	*6.5*	*9*																					
		230	150	37																					
14	11.8	5.5		14.5				32																	Died on 8th day; hemorrhages from G. I. tract.
		6		*¼.5*																					
		43		43			43	43																	
72	10.4	5.5	13	15	32	70	240	200																	Bleeding on 5th day; dead on 7th day.
		7.5	*12*	*18*	*25*	*11*	*21*	*29*																	
		29	29	29	29																				
73	14.2	5.5	8.5	12.5	22	30	120	180	300	60	900?	25	17.5	9	5.5										Recovered.
		10	*11*	*15.5*	*18.5*	*20*	*27*	*26*	*29*	*21.5*	*2¼.5*	*16*	*15*	*12*	*10*										
		21	21	21	21																				

* FIRST ROW: Prothrombin time in seconds. † SECOND ROW (*italics*): Coagulation time in minutes. ‡ THIRD ROW: Dose mg. per kilo.

Dog 6, after the development of hemorrhage, recovered and Dog 11 died after showing marked prolongation in coagulation.

The death of these animals could usually be predicted 2 or 3 days in advance. They became listless, had dyspnea, tachycardia and fever, and gave evidence of hemorrhage internally, externally or both. Tarry stools were often observed. The symptoms of a single massive fatal dose given orally or intravenously were different and will be discussed below.

Postmortem examination was made of 25 dogs, including gross and microscopic examination. Hemorrhage at some site was demonstrable in all dogs. In all cases there was subcutaneous and intramuscular hemorrhage. Frequently there was gross hemorrhage in the gastro-intestinal tract and in the pleural spaces, and multiple small hemorrhages of the lungs. In no instance was there demonstrated, grossly or microscopically, significant parenchymatous disease of the liver, kidney, or other organs. Occasionally, there was evidence of moderate hydropic degeneration of the liver but nothing more. There was, however, in most of the animals that received fatal doses, marked dilatation of capillaries, small arteries and veins. This was especially marked in those animals that died acutely from massive doses. No lesions in the walls of the vessels were demonstrable. This vascular dilatation was widespread in all viscera and was very striking. It impressed us greatly, since it was also observed, sometimes in lesser degree, in animals receiving non-fatal doses that were sacrificed for pathologic study.

The average oral dose per kilogram administered to the 6 dogs that died varied from 71 to 282 mg. per kilo. It has been repeatedly demonstrated that the fatal oral dose varies considerably and that a single massive dose is less likely to kill than are divided doses. This is exemplified in Dog 15 of Table 2, which received 5000 mg. (415 mg. per kilo) and yet survived with only moderate prolongation of the prothrombin time. Dog 72 of Table 1, on the other hand, died after a total dosage of only 1200 mg. (116 mg. per kilo), given in four divided doses. However, small doses of 3,3′-methylenebis (4-hydroxycoumarin) can be given for long periods without apparent toxicity. Thus 2 dogs of 14 and 15 kilos each received 10 mg. of the material daily for 26 days without symptoms or prolongation of the prothrombin time, indicating that there is not a large factor of cumulation in subminimal effective doses.

In Table 2 is shown the effect of oral administration of single doses of 3,3′-methylenebis (4-hydroxycoumarin) upon the prothrombin time of dogs. The doses were quite variable, and the maximal dose of 5000 mg. to Dog 1 produced death in 15 hours, the dog demonstrating marked dyspnea, convulsions, coma, and hyperthermia before death but no hemorrhagic manifestations during life or at autopsy. It will be noted that there is, within limits, some correlation of prothrombin time prolongation with the size of the dose of

* First Row: Prothrombin time in seconds. † Second Row (*italics*): Coagulation time in minutes. ‡ Third Row: Dose mg. per kilo.

the dicoumarin, as will be shown later. However, there may be marked exceptions to this rule. Doses as small as 2 mg. per kilo produced significant prolongation of the prothrombin time and a dose of 90 mg. per kilo by mouth in Dog 2 produced gross prolongation of prothrombin time, to 300 seconds. In smaller single doses by mouth, 3 to 8 mg. per kilo in dogs, significant changes in the prothrombin time were routine, but significant changes in the coagulation time could be demonstrated only occasionally.

TABLE 2.—EFFECT OF SINGLE ORAL DOSES OF 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN) UPON PROTHROMBIN TIME IN DOGS.
(Prothrombin time in seconds.)

Dog No.	Oral dose per dog, mg.	Dose per kg., mg.	Days: 0	1	2	3	4	5	6	7	8	9	10	11
1	5000	300	5.5*											
2	50	3	5.5	9	15	14.5	9.5	7	5.5	5.5				
2†	1500	90	5.5	10	38	16?	300	150	17	7.5				
3	100	7.8	6	8	16.5	11	20	16	15.5	9	7	8	6	
4	200	11.5	6	15	24		15?							
4†	250	13	6.5		16	24.5‡								
15	5000	415	6	10	15.5		20							8
41	50	7	6	8	18.5		9.5		6					
44	50	5	6	7.5	12		5.5							
45	50	1.4	5.5		11	9	6.5							
46	50	2	6		9.5	9	7							
47	50	3.3	5.5		10	13	7.5							
48	50	2.5	5.5		8	8	5.5				6			
52	50	3.5	5.5		10	8	7							
53	50	12.5	6		10		7							
54	50	3.3	6		11.5	9.5	7							

* Dead in 15 hours (no hemorrhage).
† Interval of 2 months between first and second experiment.
‡ Killed for pathologic study.

In no dog other than No. 1 of this group were symptoms of any type demonstrable, as a result of administration of the dicoumarin.

During the course of the experiment 6 dogs (Nos. 41, 44, 45, 46, 47 and 48) received 2 mg. of Proklot-Lilly (2 methyl-1, 4-naphthoquinone) by mouth daily, the first dose being given the day the dicoumarin was administered. Dogs 52, 53 and 54 received 1 mg. of Synkamin-Parke-Davis (4 amino-2 methyl-1 naphthol) intramuscularly daily. Neither the oral nor the intramuscular administration of the vitamin K appeared to have any effect whatever upon shortening the prothrombin time which had been prolonged by the dicoumarin.

Intravenous Administration of the Dicoumarin. The administration of the disodium salt of 3,3′-methylenebis (4-hydroxycoumarin) intravenously has been carried out in 21 dogs. Single doses varying from 0.5 to 50 mg. per kilo were given. Doses of 35, 40 and 50 mg. per kilo were each given to 2 dogs, and all 6 dogs died within 18 hours after administration. Before death, the dogs became

dyspneic and comatose. Fever as high as 110° has been recorded. None demonstrated hemorrhage before death or at autopsy. One animal that received 20 mg. per kilo died on the 7th day after the dicoumarin was given, from hemorrhages in the small and large bowel, but one animal survived a dose of 31 mg. per kilo with only transient symptoms and without evident hemorrhage.

TABLE 3.—EFFECT OF INTRAVENOUS DOSES OF 25 MG. PER KILO OF 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN) UPON THE PROTHROMBIN TIME AND COAGULATION TIME OF DOGS.

Dog No.	Wt., kilo.	Total dose, mg	Days: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
74	14.2	355	5.5*	9	13	22	33	35	12.5	8	6.5	7	7.5	5.5			
			11.5†	*47*	*9*	*17*	*21*	*17*	*19*	*11*	*6.5*	*16.5*	*12.5*	*7.5*			
75	10.2	255	5.5	12	13	130	145	900	240	60	45	45	8	6	6	6.5	5.5
			9.5	*9*	*11*	*17*	*29*	*22*	*21*	*20*	*15.5*	*57*	*8*	*9.5*	*9.5*	*9*	*6.5*
76	13.2	330	5.5	9	16.5	24	39	26	120	40	150?	12.5	30?	10	10	7.5	5.5
			6.5	*11*	*11.5*	*14*	*21*	*16*	*16*	*16*	*12*	*11.5*	*7.5*	*6*	*9.5*	*6.5*	*5.5*

* FIRST ROW: Prothrombin time in seconds. † SECOND ROW (*italics*): Coagulation time in minutes.

Doses of 1 to 2 mg. per kilo characteristically increased the prothrombin time from 5 or 5.5 seconds to a maximal of from 9 to 11.5 seconds on the second day after administration, and invariably the prothrombin time was normal on the 4th day. Larger doses produced a more profound and sustained effect. The effect upon the prothrombin time and coagulation time of administering 25 mg. per kilo to 3 dogs of a single experimental group is shown in Table 3. In none of these dogs were there symptoms other than slight dyspnea for a few hours. It appears from these and other experiments that 25 mg. per kilo is close to the maximal intravenous dose for dogs.

Intravascular Clot Formation. It has been demonstrated that the decrease in prothrombin in humans as a result of vitamin K deficiency was only occasionally associated with a prolongation of coagulation time until the level of prothrombin was dangerously low, 30% of normal or less. Hence it was our feeling that it might be unsafe to administer this hemorrhagic agent to man in dosages that would produce reduction of prothrombin concentration to less than 40% to 50%, nor to reduce it sufficiently to cause prolongation of the coagulation time. It was believed, however, that if the prothrombin was reduced to about 50% of normal, and maintained at this level, intravascular clotting migh. be retarded even though clot formation in a test tube was not prolonged. Hence studies were made in an attempt to demonstrate that intravascular clotting could be prolonged by the administration of 3,3′-methylenebis (4-hydroxycoumarin) in doses which appeared to us to be safe for

therapeutic purposes. Several methods of producing and studying the clot formation, such as traumatizing the vascular wall, inserting a cannula between artery and vein, and others were tried without success. The studies here reported were done by the following method:

The external jugular veins of the dog were exposed under morphine and nembutal anesthesia, care being taken to ligate all small branches. With vessels completely free, rubber-padded clamps were applied about 3 inches apart, the vein emptied of blood, and 2 cc. of monolate (monoethanolamine oleate) Abbott injected into the lumen between the clamps with a 26-gauge needle and allowed to remain for 3 minutes. The clamps were then removed to allow

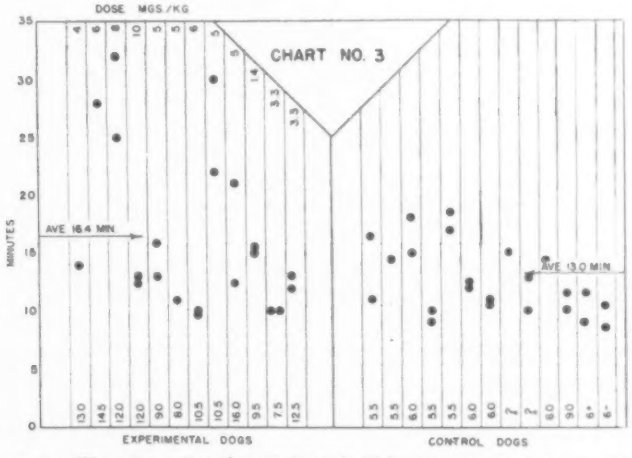

CHART 3.—The effect of 3,3′-methylenebis (4-hydroxycoumarin) upon the intravascular clotting time in dogs. The material was administered orally 48 to 96 hours prior to the experimental observations. Each vertical column represents an experiment and each dot represents the time of clot formation in one vessel. The prothrombin time in seconds in each experiment is indicated by the figures in the respective columns.

blood to wash through the veins for 1 minute. Five minutes after reapplication of the clamps, the vessel was gently palpated at intervals of 1 minute to check upon the formation of a clot. This method of detection gave quite consistent results when checked with the opposite vein immediately afterwards. The vessels were always opened to check the palpatory findings and sometimes opened when no clot was felt to check the technique. Palpation of the second side was repeated at less frequent intervals to keep the manipulative factors at a minimum. Even with this refinement, it is obvious, however, that the experiment is crude and includes variables beside the monolate which will hasten clotting.

The results of these experiments are shown in Chart 3.

Observations on Humans. From the first, despite the apparent high tolerance and high therapeutic index of 3,3′-methylenebis (4-hydroxycoumarin) for dogs, we approached the problem of its administration to humans with temerity. The first 12 patients to receive the material were, with but 1 exception, patients with advanced malignancy. Only 1 demonstrated definite clinical evidence of involvement of the liver.

TABLE 4.—EFFECT OF 3,3′–METHYLENEBIS (4–HYDROXYCOUMARIN) ADMINISTERED INTRAVENOUSLY TO PATIENTS.

Pt.	Sex.	Diagnosis.	Wt., kg.	Dose, mg./kg.	Days: 0	1	2	3	4	5	6	7	8	9
S. C.	M	Peptic ulcer	74	1 7	12*	15 5	15 5	13 5	15 5	12	11 5	12	12	12
					11	*15*	*18*	*11*	*15*	*12 5*	*12*	*15 5*	*14 5*	*7 5*
G. G.	F	Bronchiogenic carcinoma	60	2 0	12	16	16,5	14 5	Discharged					
					5 5	*12*	*14*	*8 5*						
O. N.	M	Arteriosclerosis; asthma	70	3 0	12	15	16	15	18	16	12	12	12	12
					9 5	*16*	*21*	*28*	*28*	*13 5*	*12*	*12*	*16*	*12 5*
A. W.	M	Arteriosclerosis	73	4 0	12	18	22 5	22	13	12	12			
					10 5	*8 5*	*18*	*16*	*8 5*	*15*	*11*			
A. L.	M	Psychoneurosis	66	4 0	12	14	17	15	11 5	12				
					7	*13*	*7 5* ?	*14*	*7 5* ?	*15 5*				
D. B.	M	Silicosis	80	4 0	11 5	15	22 5	24	14	12	12			
					8 5	*14*	*18 5*	*16*	*14*	*15*	*13 5*			

* Control time for this particular supply of thromboplastin was 12 seconds. Dilutions of normal plasma to 50% gave a time of 16.5 seconds; dilution to 40% gave a figure of 23 seconds with this thromboplastin.

† FIRST ROW: Prothrombin time in seconds.

‡ SECOND ROW (*italics*): Coagulation time in minutes.

In all observations on humans complete blood counts, urinalyses, and blood sugar determinations were made. For several the hippuric acid liver function test, the icterus index, and the cephalin-cholesterol test of Hanger,[10] complete blood counts and platelet counts, bleeding and coagulation times were done before the dicoumarin was administered. In no instance were gross changes in these findings observed after administration of the dicoumarin. All these patients received the material in gelatin capsules, orally. The first patient received 10 mg. of the dicoumarin on the 1st, 3d and 5th days, 25 on the 6th, and 50 mg. on the 10th day of observation. The other 11 patients, with 1 exception, received single doses ranging from 50 to 100 mg. (0.75 to 2 mg. per kilo). In 5 of the 11 patients the prothrombin time was prolonged from an average control level of 10 seconds to an average maximal level of 12 between the 2d and 6th day after the material was administered. These changes, while not marked, appear to us to be significant, since the doses were small. Six patients to whom similar dosages were admin-

istered showed no prolongation in the prothrombin time. In none of the 12 patients was there the slightest clinical evidence to indicate that the dicoumarin was unfavorably borne. Butt, Allen and Bollman[2] have given human beings much larger doses than we have administered without the development of toxic symptoms.

The disodium salt of 3,3′-methylenebis (4-hydroxycoumarin) was given intravenously to human beings because of the apparent variation in absorption when it was given orally. It was thought that the effective therapeutic dose might thus be more readily established. To date this material has been given to 10 human beings in doses ranging from 0.5 to 4 mg. per kilo without the development of any toxic symptoms. The effects of the larger doses upon the prothrombin time and coagulation time for 6 of these are shown in Table 4. The first 4 cases are omitted because the doses were smaller, 1 mg. per kilo or less, and produced no discernible effects. The maximal therapeutic dose and the optimal effective dose is not as yet established.

Complete blood counts with platelet estimations, repeated urine analyses, blood sugar and non-protein nitrogen determinations, and icterus indices were done on all patients at the beginning and end of the experimental period. Hippuric acid and cephalin cholesterol determinations were made on 4 of the patients before and after the experimental period. No significant changes were noted.

The results show a distinct effect upon both the prothrombin time and the coagulation time. The control levels of prothrombin in this particular group of experiments are higher than usual for human beings with the technique we use, but can be explained by the fact that the thromboplastin of this particular time was somewhat less potent than that which we usually made. This variation in thromboplastin occasionally occurs, even when it is fresh, and thus the need for control observations.

It would seem from these results that rather small doses, 100 to 300 mg. (2 to 4 mg. per kilo) are sufficient to prolong the coagulation time very significantly. Some might even consider these levels hazardous, for the possibility of internal or intracranial hemorrhage must be considered. On the other hand, the results strongly suggest the possible efficacy of the material in preventing thromboses.

The variation in the effects resulting from doses of different sizes is striking. For example, 3 mg. per kilo in Patient O. N. produced a more profound effect upon the coagulation time, but not upon the prothrombin time, than did doses of 4 mg. per kilo given to Patients A. W., A. L. and D. B. This observation calls for considerable thought and emphasizes that the proper and safe dosage is yet to be worked out. It also emphasizes the need for considerable caution in prescribing the dicoumarin until further data are available.

In Vitro Experiments. The possibility that the 3,3′-methylenebis (4-hydroxycoumarin) might prolong the prothrombin time *in vitro*

was considered. To exclude this possibility, in both dogs and human beings, oxalated plasma of 2 dogs and 12 humans was obtained. To 0.1 cc. of the plasma of each was added 0.1 cc. of water and to a second, third and fourth set of test tubes containing 0.1 cc. of the plasma of each, was added 0.1 cc. solution of dicoumarin of three different levels of pH, 7.3, 7.8 and 8.5. The solutions were then incubated for periods of from 2 minutes to 3 hours. Although the prothrombin time increased with the longer incubation periods, from 10 to 25 seconds in some instances, the prolongation in the controls, where only water had been added, was as great in every experiment. It must, therefore, be concluded that this dicoumarin does not prolong the prothrombin time in dog or human plasma *in vitro*. This confirms the observations of Link and his coworkers.[4,7,12]

Discussion. It is evident that 3,3′-methylenebis (4-hydroxycoumarin) synthesized by Stahmann, Huebner and Link is fully capable of producing prolongation in the prothrombin time and coagulation time in dogs and humans. The material is effective when administered orally or, as the disodium salt, intravenously. The prolongation in prothrombin time is not effective until 24 hours after administration of the material by mouth, and there is a similar but perhaps slightly shorter latent period with intravenous administration. For some days, varying with the dose, the prothrombin time becomes increasingly prolonged, and then, if no more than the single dose is given, gradually returns to normal, usually between the 5th and 7th day when small doses are given, by the 7th to 10th when large doses are given. Whatever the size of the dose, the initial latent period was always observed and during the first 2 days the prolongation of prothrombin times were approximately the same. Only after this period did the larger doses exhibit the greater depression of prothrombin activity. Divided doses given orally were more effective in reducing the prothrombin than was the same quantity given in a single dose; this suggested that above a certain level there is some difficulty with absorption of the material. Link[12] and his coworkers have reported recovery of considerable quantities of the dicoumarin in the feces of animals given massive doses by mouth.

The ideal dosage of the dicoumarin for human beings is not yet established, but it may be said, on the basis of studies in dogs, that the maximal tolerated dose when given intravenously is almost certainly not much greater than 25 mg. per kilo and perhaps less. From our experiments it was suggested that doses of 2 to 4 mg. of the disodium salt given intravenously are probably safe; such doses prolong the prothrombin time to about 40% to 50% of normal and usually prolong the coagulation time significantly. Similar dosage by mouth is probably also adequate. The timing of repeated doses is yet to be worked out by many observations; for the present they should be individualized by daily determinations of the prothrombin time and coagulation time. Butt, Allen and Bollman[2]

have given single doses of 1000 mg. orally, apparently without resultant toxicity.

It is suggested, but not established by experiments in dogs, that intravascular clotting may be prolonged by the previous administration of the dicoumarin in moderate dosage. Further study of this problem is contemplated but it is technically a difficult one, and an ideal method of studying intravascular clotting is difficult to attain.

The treatment of toxic symptoms or signs is confined at present to the control of prothrombin and coagulation times. From the experiences of Schofield, Roderick, Butt, Allen, and Bollman and ourselves, transfusions of serum or whole blood will cause a prompt lowering of prothrombin and coagulation times. This effect is not sustained and may have to be repeated if the coagulation time returns to dangerous levels.

The frequent observation of profound capillary dilatation that follows administration of dicoumarin, as demonstrated at autopsy, even when only moderate doses are given, raises the question of a gross vascular factor in the hemorrhagic manifestations of sweet clover disease in cattle. This factor as a possible contributing mechanism in the production of undesirable bleeding when the dicoumarin is used clinically must be borne in mind.

The mechanism by which the dicoumarin prolongs the prothrombin time, and hence the coagulation time, is not known. Even when fatal doses were given to dogs, there were no demonstrable gross or microscopic changes in the liver which could be attributed to the substance administered. It might be hypothesized that the formation of prothrombin in the liver is physiologically inhibited, and that only when the prothrombin present in the blood at the time of administration of the dicoumarin is used up, is there demonstrable prolongation in the time. This would furnish an explanation for the usual latent period of 24 hours or more before the prothrombin time is increased. The possibility that the function of prothrombin formation by the liver is inhibited is strengthened by the fact that vitamin K administration in adequate dosage is ineffective in preventing the increase in prothrombin time. Other possibilities to explain the effect of 3,3′-methylenebis (4-hydroxycoumarin), of course, come to mind, although experimental evidence for them also is lacking. Thus the material may, during the latent period, undergo change* of some type in the body before it is capable of functioning; or the prothrombin produced in the presence of the hemorrhagic agent is altered in such a way that it is inactive; or immediately after administration a protective mechanism may be set up *in vivo* which prevents the toxic substance from affecting the prothrombin. The

* Studies underway by W. R. Sullivan, R. S. Overman, M. A. Stahmann and K. P. Link indicate that the lag period is most likely due to the fact that the effect of the dicoumarin does not become apparent until it has undergone change in the animal organism.

possibility that the hemorrhagic agent inactivates the vitamin K in the body, and even the vitamin K administered subsequent to administration of the dicoumarin cannot be excluded as a cause for the decrease in prothrombin. This material is ineffective in prolonging the prothrombin time in plasma *in vitro*.

The possible importance of 3,3′-methylenebis (4-hydroxycoumarin) as a substitute for heparin in the prevention of thrombosis is self-evident. The material can be readily prepared at a reasonable cost. It can be given orally or intravenously with prolonged effect. It is possible that it may, in the future, replace heparin. But much is yet to be done. Our knowledge is still of an experimental nature and we feel that its indiscriminate use may be very hazardous, even though the therapeutic index appears to be high. It is urged that those who employ this material continue to select the patients carefully, avoiding those with gross hepatic disease, and that for the present the apparent minimal effective doses be used. It is our plan to continue the study of this interesting substance in the hope of establishing its safety and its value in the prevention and treatment of thrombosis. Its possible advantages in the prevention of adhesions, and in vascular surgery and other situations where heparin has been used remain to be investigated.

Conclusions. The synthetic dicoumarin 3,3′-methylenebis (4-hydroxycoumarin) when administered orally or intravenously produced, after a usual latent period of 24 hours or more, prolongation of prothrombin time and coagulation time in dogs and human beings.

Therapeutic and fatal doses of this material given to dogs did not produce significant pathologic changes in the liver or other parenchymatous organs but did, in many instances, produce capillary, arteriolar and venule dilatations.

No harmful effects were observed from the administration of the dicoumarin to humans.

The administration of synthetic vitamin K did not shorten the prothrombin time previously lengthened by administration of the dicoumarin, but transfusion of blood was temporarily effective in shortening the prothrombin time and coagulation time after they had been prolonged.

This dicoumarin may well prove to have an important place in clinical medicine as a substitute for heparin in the prevention and treatment of thrombosis and in other conditions.

Dr. John C. McCarter examined the pathologic sections for us and Miss Emily Gray prepared the tissues. We wish gratefully to acknowledge their valuable aid.

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A GLOBULIN-FRACTION IN RABBIT'S PLASMA POSSESSING A STRONG CLOTTING PROPERTY.

By I. A. Parfentjev, Ph.D.,
PEARL RIVER, N. Y.

(From the Department of Biological Research, Lederle Laboratories.)

In the course of work on the purification of rabbit plasmas it was observed that a certain fraction obtained from these plasmas was capable of producing clotting when added to plasma or blood. A description of some investigations into the nature of this substance with a summary of our findings is given in this paper.*

Method. A study was made of several fractions of rabbit plasma prepared by successive precipitating with ammonium sulphate.† The first fraction was that precipitated by the addition of 200 gm. of ammonium sulphate crystals to each liter of diluted rabbit plasma. The precipitate was twice redissolved in distilled water in one-half of the original volume and reprecipitated at about 20% ammonium sulphate. The final precipitate was dialyzed for 2 days in tap water; this fraction was designated *I-W*. In some cases an acid-insoluble portion was removed from the practically salt-free dialysate. Acidification of the dialysate to pH 5.3 with normal lactic acid and dilution with distilled water if indicated by preliminary test, generally removed all water-insoluble proteins. Occasionally a second, but relatively small, fraction of water-insoluble protein could be eliminated by subsequent adjustment to pH 5.8. These water-insoluble proteins were suspended in 1% salt solution at neutrality; they were designated *I-WI*. The water-soluble proteins were adjusted to neutrality, 1% sodium chloride was added, and the material was concentrated by ultrafiltration. This fraction was identified as *I-WS*.

The second fraction was precipitated by the addition, to each liter of filtrate after removal of the first fraction, of 100 gm. of crystalline ammo-

* A preliminary report on this subject was published in Science, **93**, 328, 1941.

† We are indebted to Mr. James Jacques for technical assistance in this fractionation.

nium sulphate. As in the case of the first fraction, the precipitate thus obtained was twice redissolved in distilled water and reprecipitated at 30% ammonium sulphate. After that the precipitate was dialyzed for 2 days in tap water; it was identified as *II–W*. Further separation into water-insoluble (*II–WI*), and water-soluble (*II–WS*), fractions was effected in a manner similar to that employed with the first fraction.

Addition of 200 gm. of crystalline ammonium sulphate to each liter of filtrate after removal of the second fraction precipitated the third fraction. This made a total of 500 gm. of ammonium sulphate per liter of original plasma. All the thermolabile proteins of rabbit plasma are precipitated at this concentration of ammonium sulphate. This third precipitate was dialyzed for 2 days in tap water; it was identified as *III–W*. Acidification of the dialysate of this fraction to pH 5.3 yields a very small water-insoluble fraction (*III–WI*). The greater part of the proteins of the third fraction is water-soluble (*III–WS*). This third fraction contains the greatest portion of the proteins of rabbit plasma.

Many batches of rabbit plasma were fractionated in this manner, and the fractions were studied for their clotting power.

Experiment With Plasma. To 0.9 cc. of the plasma in a Wassermann tube was added 0.1 cc. of the material under test. The tube was then placed in the water-bath at 37° C., and the clotting time was recorded.

Preliminary experiments have consistently demonstrated that the precipitate obtained from rabbit plasma at between 20% and 30% (200 and 300 gm. per liter) of ammonium sulphate possesses practically all of the clotting power of the original plasma. This fraction (*II–W*) will be referred to as clotting globulin. More detailed study showed that a considerable portion of the clotting factor could be precipitated with the water-insoluble proteins *II–WI* at pH 5.3 in the absence of salt.

TABLE 1.—THE CLOTTING OF HORSE PLASMA CONTAINING 1% SODIUM CITRATE BY DIFFERENT FRACTIONS OF RABBIT PLASMA.

Dilutions of test material with normal horse citrate plasma	Fraction *I–W*.		Fraction *II–W*.		Fraction *III–W*.	
	Water soluble *I–WS*. NR9–1. Solids 12.1%.	Insoluble at pH 5.2 *I–WI*. NR9–4. Solids 13%.	Water soluble *II–WS*. NR9–2. Solids 12%.	Insoluble at pH 5.2 *II–WI*. NR9–5. Solids 7.3%.	Water soluble *III–WS*. NR9–3. Solids 12.7%.	Insoluble at pH 5.2 *III–WI*. NR9–6. Solids 1.7%.
	Figures indicate time, in minutes, within which clotting occurred.					
1:10	—	—	30	Less than 1	—	—
1:100	—	—	30	Less than 1	—	—
1:200	—	—	60	Less than 1	—	—
1:500	—	—	60	Less than 1	—	—
1:1000	—	—	—	8	—	—
1:2000	—	—	—	60	—	—
1:5000	—	—	—	60	—	—
1:10000	—	—	—	—	—	—

All fractions were prepared from normal rabbit plasma.
Failure to clot within 24 hours is indicated by a dash (—).

Horse, rabbit and human plasmas were secured by drawing blood from the vein into vessels containing potassium oxalate solution, in the proportion of 9 cc. of whole blood to 1 cc. of 2.6% potassium oxalate solution. The plasma was then freed of red cells by centrifugation. In a Wassermann tube, 0.9 cc. of a given plasma was mixed with 0.1 cc. of rabbit fraction number 356–6, in varying dilution; the mixtures were then placed in the water-bath at 37° C. and the clotting times were noted. This material (No. 356–6) represents the second fraction of rabbit plasma; more specifically, that part of rabbit plasma which is insoluble in water at pH 5.2 (*II–WI*). The content of solids of this material was 16.6%. All three plasmas, from the horse, the rabbit and man were clotted by the material under test.

TABLE 2.—THE CLOTTING OF THE PLASMA FROM DIFFERENT SPECIES OF ANIMALS BY THE SECOND FRACTION ISOLATED FROM RABBIT PLASMA (SPECIMEN NO. 356–6).

Dilution of test material. with plasmas	Normal horse plasma.	Normal human plasma.	Normal rabbit plasma.
	Time in which clotting occurred.		
1:10	5 sec.	5 sec.	5 sec.
1:100	20 sec.	10 sec.	5 sec.
1:1000	210 sec.	15 sec.	10 sec.
1:5000	24 hrs.	6 min.	4 min.
1:10000	—	60 min.	60 min.
Control*	—	—	—

* One volume of 0.85% NaCl mixed with 9 volumes of plasma.
Failure to clot within 24 hours is indicated by a dash (—).

A study was made on the clotting, by rabbit's plasma fraction *II–W*, of normal horse plasmas prepared with different anticoagulants. The anticoagulants employed were: 1, Potassium oxalate, the final concentrations in the plasmas being 0.1%, 0.5%, 1%. 2, A commercial preparation of heparin; 1 mg. of which, according to the specifications, prevents the coagulation of 5 cc. of cat blood for 24 hours. In our experiments the final concentration of heparin in the plasma was 0.05% or 0.1%; with such concentrations, horse plasma would remain fluid for more than 24 hours. 3, Germanin; final concentrations in the plasma of 0.15%, 0.2%, 0.4%.

The clotting substance used was rabbit plasma fraction *II–WI*, with a content of solids at 7.3%. The technique followed has already been described. The findings of this series of tests are shown in Table 3.

It was observed that the action of clotting globulin (*II–W*) is largely independent of the nature and concentration of the anticoagulant within the range of the substance tried. Increasing the final concentration in the plasma of potassium oxalate to as much as 1% serves only to delay, but does not stop, the clotting of normal horse plasma by fraction *II–W* of rabbit plasma. It should also be mentioned here that the clotting of normal horse plasma

containing 1% sodium citrate by rabbit plasma fraction *II–W* has been regularly observed.

TABLE 3.—THE INFLUENCE OF THE ANTICOAGULANT ON THE CLOTTING OF NORMAL HORSE PLASMA BY RABBIT PLASMA FRACTION *II–W*.

Dilutions of Fraction *II–WI* with normal horse plasma.	Potassium Oxalate.			Heparin.		Germanin.	
	Final concentration of anticoagulant in the plasma.						
	0.1%.	0.5%.	1.0%.	0.05%.	0.10%.	0.1%.	0.2%.
	Time in which clotting occurred.						
1:10	1 min.	10 min.	20 min.	1 min.	1 min.	1 min.	2 min.
1:100	1 min.	10 min.	20 min.	3 min.	3 min.	1 min.	—
1:1000	2 hrs.	24 hrs.	24 hrs.	—	—	2 hrs.	—
1:2000	—	—	—	—	—	—	—
1:5000	—	—	—	—	—	—	—
1:10000	—	—	—	—	—	—	—

The particular Fraction *II–WI* used was No. NR9–5.
Failure to clot within 24 hours is indicated by a dash (—).

Clotting globulin was observed to clot rapidly horse plasma deprived of its own prothrombin by absorption with magnesium hydroxide. Horse plasma containing 0.26% potassium oxalate was mixed with 10% of its volume of a 3% suspension of magnesium hydroxide. The mixture was shaken several times in the next 2 hours at room temperature, after which it was centrifuged and the reaction of the supernatant absorbed plasma was adjusted to neutrality. Such absorbed plasma does not clot on recalcification; however, the addition of the above described clotting globulin (*II–W*) in small quantities was able to clot it.

Experiments With Whole Blood. In this work the 15 by 150 mm. test tube, coated inside with a thin even layer of paraffin, was used. When 1 cc. of physiologic salt solution and 9 cc. of whole blood are mixed in such a tube, the average clotting time at room temperature is between 25 and 30 minutes. The introduction into such a preparation of very minute portions of rabbit fraction *II–W* considerably reduces the clotting time. Tests of this kind, using fractions No. NR9–5 and No. 356–6 on whole horse blood, are reported in Table 4.

TABLE 4.—THE CLOTTING OF WHOLE HORSE BLOOD BY FRACTION *II–W* (SPECIMENS NR9–5 AND 356–6).

Dilutions of test material with whole horse blood.	Specimen NR9–5.			Specimen 356–6.
	Exp. 1.	Exp. 2.	Exp. 3.	Exp. 4.
	Time in which clotting occurred.			
1:10	10 sec.	15 sec.	10 sec.	
1:100	40 sec.	45 sec.	5 sec.	
1:1000	1 min.	1 min., 20 sec.	20 sec.	1 min., 30 sec.
1:10000	3 min.	6 min., 40 sec.	5 min., 15 sec.	4 min.
1:100000	10 min., 25 sec.	10 min., 35 sec.	2 min., 5 sec.	18 min.
Control (1 vol. physiologic saline, 9 vols. whole blood)	30 min., 25 sec	16 min., 30 sec.	25 min.	44 min.

The Relative Toxicity of the Three Rabbit-plasma Fractions. In the fractionation of immune rabbit plasma with ammonium sulphate, as referred to in an earlier part of this paper, the major portion of the antibodies has been found in fraction *I–W*.

Tests of Toxicity in Mice. It was shown that there is a marked difference in the toxicity of the three fractions so obtained. For example, mice were not obviously affected by the intravenous injection of 0.4 cc. of fraction *I–WI*, or of fraction *III–WI*, containing 12% solids. Fraction *II–WI*, however, proved to be lethal for mice, when injected intravenously. Each of 2 mice received an intravenous injection of 0.01 cc. of fraction *II–WI*, No. NR9–4 (12% solids) prepared from normal rabbit plasma; in a few minutes both mice were dead. Similarly, when each of a series of 6 mice received intravenously 0.01 cc. of No. 13C14–4 (10% solids) a preparation of fraction *II–WI* from rabbit antimeningococcal plasma, each animal died within a few minutes.

Fraction *II–W*, when injected subcutaneously or into the abdominal cavity of the mouse is more easily tolerated. Two mice, each receiving 1 cc. subcutaneously of No. 13C14–4 survived for 2 weeks.

Tests in Rabbits. The "toxicity" of the clotting globulin was also studied in the rabbit for the purpose of determining whether the lethal effect is due to intravascular clotting. This was found to be the case.

Rabbit 1 (5 pounds) received 1 cc. clotting globulin 13–C–19 intravenously (slowly). Death occurred in 2 minutes. Autopsy immediately. Right auricle and ventricle filled with completely clotted blood, the clot extending into the pulmonary artery filling it. The left chambers of the heart contained little fluid blood.

Rabbit 2 (5 pounds) received 0.25 cc. clotting globulin 13–C–19 intravenously; not slowly. No symptoms. After 7 minutes 0.5 cc. clotting globulin 13–C–19 intravenously (opposite ear, slowly). No symptoms at any time.

Rabbit 3 ($8\frac{1}{4}$ pounds) received 1 cc. clotting globulin 13–C–19 intravenously, slowly. No symptoms in 5 minutes. Then injected another quantity of 1 cc. clotting globulin 13–C–19 into the other ear; death occurred in 2 minutes. Autopsy showed right ventricle filled with mostly fluid blood; a thin clot was found extending into the pulmonary artery in which it was deeply attached.

The Stability of the Clotting Factor Associated With Fraction II–W. In a review of a large number of batches of rabbit plasma processed up to the present, in no instance have we failed to obtain an active clotting globulin. Using the technique of testing described earlier in this paper, one volume of such a preparation may be found to clot 100 volumes of oxalated horse plasma within 30 seconds, and to clot 1000 volumes of plasma within $\frac{1}{2}$ hour. The usual preparations of fraction *II–W* have a solids content of about 10%.

Our experiments have indicated that this clotting globulin is

comparatively stable. For instance, specimen No. NR6-2 (fraction *II-W*) was kept for 6 months at about 5° C., without preservative, with no appreciable alteration of clotting titer. Several other preparations of fraction II-W, containing 0.4% phenol and 1 to 50,000 phenyl mercuric acetate, were passed through a Berkefeld filter candle. After 9 months' storage at 5° C. the material still had considerable clotting power, though the titer was somewhat reduced.

A series of experiments has shown that heating somewhat weakens the clotting factor, but does not entirely destroy it. Even after being heated at 75° C. for 10 minutes, some of this material was able to clot 10 times its volume of plasma. Preservatives enhance the deterioration caused by heat; in this respect phenol is more destructive than merthiolate, in the concentrations tested.

The clotting fraction may be frozen and thawed without alteration of its clotting power.

The proteins associated with the clotting factor may be precipitated with 70% and 90% acetone. The redissolved precipitate possesses the clotting titer of the original material.

Discussion. Leo Loeb[5a-e,9,10,11a,b,12,16] has reported that the intravenous injection of heterogenous serum may bring about the clotting of blood in the vessels. He has observed intravascular clotting in the pulmonary blood-vessels of rabbits injected with dog serum. Loewit[7] attached considerable importance to this observation, feeling that it would assist in the evaluation of the primary toxicity of serum.

Loeb's findings have been of value to us as a guide in the purification of rabbit sera. In our efforts to free immune rabbit sera of its clotting action, we isolated a small globulin fraction having a clotting power of a very high order. In the studies of the activity of this preparation we have been using the technique outlined by Quick, Smith, *et al.*[15a,b,19a,b,20-26]

The rôle of calcium in the mechanism of the clotting of blood was discussed not long ago by Howell[6] and Mellanby.[14] The clotting globulin that we have isolated from rabbit's blood, does not need calcium and has shown much strength in the presence of excess of oxalate, citrate and other anticoagulants. This clotting globulin acts directly on fibrinogen and is able to clot plasma whose own prothrombin has been absorbed with magnesium hydroxide. Preparations of this material have been proven to be comparatively stable.

From human plasma Taylor and his collaborators[13,17,18] have secured certain coagulating globulins, and other constituents of blood having clotting properties have been noted by Glazko and Greenberg[4] and Bendien and Van Creveld.[2] No comparison of these preparations with our rabbit clotting globulin has been made.

Hess[5a,b] writing about the injection of horse serum in intractable

hemorrhage pointed out that the same acceleration of coagulation could be achieved by injection of the purified euglobulin fraction instead of whole serum. The author obtained the euglobulin fraction from human, horse and sheep plasma by fractionation with ammonium sulphate. The concentration of ammonium sulphate that he used for precipitation of euglobulin corresponds to that by which we obtained our fraction *I–W* from rabbit's plasma. He claimed a favorable clinical effect of such an injection. However, the tables given in this paper on experiments carried out *in vitro* revealed quite a low clotting capacity of his euglobulin fraction.

Our observations on fraction *II–W* have naturally suggested the use of this fraction as a hemostat. Work is now in progress on a study of the value of this preparation as compared with other hemostatic agents which have recently been reviewed by Aggeler and Lucia[1a,b] and Ferguson.[3]

Conclusions. 1. A description is given of a globulin fraction, isolated from rabbit plasma which possesses the property of clotting plasma.

2. It has been found that the "toxicity" of this fraction on intravenous injection is due to intravascular clotting.

3. Clotting globulin could be separated from that fraction of rabbit plasma which contains the major portion of the immune bodies.

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THE THROMBIC ACTIVITY OF A GLOBULIN FRACTION DERIVED FROM RABBIT PLASMA.

By F. H. L. Taylor, Ph.D.,

RESEARCH ASSOCIATE IN MEDICINE, HARVARD MEDICAL SCHOOL; CHEMIST, THORNDIKE MEMORIAL LABORATORY, BOSTON CITY HOSPITAL,

Eugene L. Lozner, M.D.,

ASSISTANT IN MEDICINE, HARVARD MEDICAL SCHOOL; RESIDENT PHYSICIAN, THORNDIKE MEMORIAL LABORATORY, BOSTON CITY HOSPITAL,

AND

Margaret A. Adams, B.A.,

LABORATORY ASSISTANT, THORNDIKE MEMORIAL LABORATORY, BOSTON CITY HOSPITAL, BOSTON, MASS.

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.)

Parfentjev has reported[9a] that a globulin which possesses the property of coagulating citrated blood may be obtained by the fractionation of the proteins of rabbit plasma with ammonium sulphate. The present communication presents evidence confirming this observation and attempts to identify this clot-promoting factor in the light of modern knowledge of blood coagulation. In the accompanying paper[7] certain practical clinical applications of this material will be presented.

Experimental. *Methods.* Rabbit plasma was obtained through the courtesy of the Lederle Laboratories and fractionations were carried out as suggested by Parfentjev[9a,b]. Occasionally slight modifications in the procedure were made for purposes of comparison and quantitation.

Five hundred milliliters of rabbit plasma* obtained from rabbit whole blood containing as an anticoagulant 2.5% sodium citrate, were diluted with an equal volume of 0.9% sodium chloride solution. To 1 liter of the diluted plasma, 200 gm. of Merck's anhydrous ammonium sulphate were added and the pH of the mixture was adjusted to 7.4 (using phenol red indicator) with 1 N. sodium hydroxide. The mixture was then stirred mechanically at 37.5° C. for 16 to 18 hours. The resulting precipitate of plasma protein was removed by filtration. The filtrate was retained.

The precipitate was removed from the filter paper and redissolved in 500 ml. of 0.9% sodium chloride solution. To this, 100 gm. of ammonium sulphate were added. The pH was again adjusted to 7.4. The mixture was stirred at 37.5° C. for from 3 to 4 hours and filtered. The filtrate was combined with that from the first precipitation. The precipitate was again redissolved and the procedure of reprecipitation carried out as before. The final precipitate was discarded.

The filtrates from the three precipitations were pooled and the volume was measured. Sufficient anhydrous ammonium sulphate was added to raise the concentration of this salt to 30%. The pH was adjusted to 7.4. The mixture was allowed to stand at room temperature for 72 hours. During this time the precipitate settled out completely. The supernatant

* The Lederle Laboratories, Inc., have advised us that the plasma as received by us contains approximately an equal volume of 2.5% sodium citrate as the anticoagulant solution.

fluid was removed as completely as possible by syphonage and the remaining small amount by filtration.

The precipitate was removed from the paper and dissolved in a volume of 0.9% sodium chloride solution equal to one-tenth of the previous large volume. Thirty grams of anhydrous ammonium sulphate were added for each 100 ml. of sodium chloride solution used, and the pH was adjusted to 7.4. The mixture was stirred at 37.5° C. for from 4 to 5 hours and filtered. The filtrate was discarded. The precipitate was removed from the paper and dissolved in the minimum amount of distilled water necessary to effect solution. This was transferred to cellophane sacks and dialyzed against running tap water at approximately 7° C. for 48 hours.

The contents of the cellophane sacks were transferred to weighed serum bottles and dried by the lyophile method.[4] The yields of dried material from 500 ml. of rabbit plasma ranged from 200 to 1400 mg. For purposes of quantitation the dried contents of the serum bottles were taken up into sufficient amounts of 0.9% sodium chloride solution to give it a concentration of total solids of 12%.

Certain modifications in the procedure were occasionally made for various reasons as indicated below.

Thrombic Activity of Rabbit Pseudoglobulin. Parfentjev has reported that his preparation of pseudoglobulin coagulated citrated blood. An attempt was therefore made to study the precipitation of fibrinogen by this material quantitatively. In the following experiments a 1:100 dilution of the sodium chloride solution of the rabbit globulin fraction was employed. Citrated normal human plasma was used as a source of fibrinogen in all experiments. In most of these 1 ml. of plasma was pipetted into each of a series of beakers and diluted with 30 ml. of 0.9% sodium chloride solution. Occasionally the amount of plasma was doubled when more fibrinogen was required for the experiment. The fibrinogen content of the plasma was determined in one sample by a method of recalcification.[3] To the remaining samples of diluted plasma, varying amounts of the diluted pseudoglobulin preparation were added and the amount of fibrinogen, precipitated as fibrin, was determined. This determination was made both by the method of nitrogen difference and also by the direct determination of the nitrogen of the precipitated fibrin.[3]

For purposes of comparison the amount of nitrogen in each pseudoglobulin preparation was determined and the number of milligrams of fibrinogen converted to fibrin was plotted against the amount of globulin nitrogen added.

Table 1 shows the quantitative data from one such experiment. Comparable data were obtained on 8 other preparations of rabbit pseudoglobulin. These are shown graphically in Chart 1.

TABLE 1.—THE RELATIONSHIP OF RABBIT THROMBIN NITROGEN TO THE AMOUNT OF FIBRINOGEN CONVERTED TO FIBRIN FROM 1 ML. OF CITRATED NORMAL HUMAN PLASMA.

Mg. rabbit thrombin nitrogen added to plasma.	Mg. fibrin precipitated.
0.01	1.54
0.02	1.89
0.03	2.25
Total fibrinogen present in plasma as determined by recalcification	2.23

Since citrated plasma was used as a source of fibrinogen in these experiments, the precipitation of the fibrinogen might have been attributed to one of two possible reactions. If the preparation of rabbit pseudoglobulin contained sufficient calcium, then any thromboplastin in the preparation acting by releasing thrombin from the prothrombin of the plasma, could cause conversion of fibrinogen

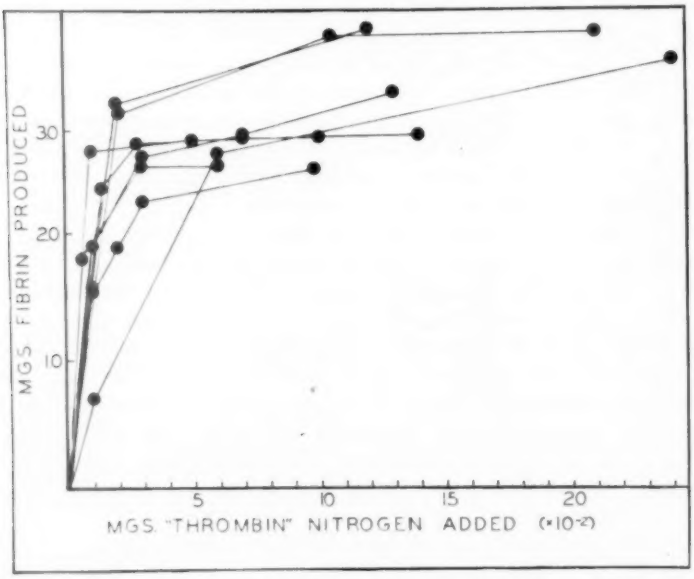

CHART 1.—The quantitative relationship of the amount of plasma fibrinogen converted to fibrin to the amount of rabbit thrombin added.

to fibrin. Or this conversion might have been the result of the direct action of thrombin independently of the other factors in the blood coagulation reaction. The calcium content of the various pseudoglobulin preparations was determined by a modification of the method of Fiske and Subbarow and related to their nitrogen content. As will be observed from the data presented in Table 2,

TABLE 2.—THE RELATIONSHIP OF CALCIUM TO NITROGEN CONTENT OF VARIOUS PREPARATIONS OF RABBIT THROMBIN.

Preparation No.	Mg. nitrogen per gm. of total solids.	Mg. calcium per gm. of total solids.
1	92	1.06
2	103	0.51
3	107	1.14
4	111	0.49
5	119	0.69
6	118	0.79
7	112	0.94
8	120	0.36

all the preparations contained small amounts of calcium which apparently were carried through the entire procedure. In no instance, however, did any of the preparations contain sufficient calcium to initiate coagulation. For example, on the basis of the data of Table 2, the largest amount of calcium added to the 1 ml. of plasma in the series of observations presented in Table 1, was 0.0001 mg., which could have had no effect on the conversion of fibrinogen to fibrin in 1 ml. of citrated plasma.

It would appear from an examination of the procedure that the clotting activity of the rabbit plasma was associated with the pseudoglobulin fraction. In view of the fact that the thromboplastic activity of human plasma has been shown to reside almost wholly in the euglobulin fraction,[6,10] observations were made on rabbit plasma following the removal of euglobulin by dialysis of the plasma against cold running tap water for 8 days. Following this dialysis the rabbit euglobulin was found to resemble that from human plasma in that it contained almost all the coagulant activity of the plasma for hemophilic blood. Yet, when the remaining plasma proteins were treated with 30% ammonium sulphate and the latter part of Parfentjev's procedure carried out, a pseudoglobulin could be prepared which clotted citrated plasma. These data are presented in Table 3. This evidence indicates therefore that the clot-promoting substance in rabbit pseudoglobulin is neither calcium nor thromboplastin nor both calcium and thromboplastin. By definition therefore the clotting activity of rabbit pseudoglobulin must be the result of true thrombin and hereafter this rabbit fraction shall be referred as "*rabbit thrombin.*" Such a conclusion is further justified by observations that the material is active both in the presence of large excesses of citrate or oxalate in the plasma after prothrombin is removed from the plasma by adsorption, and on fibrinogen solutions.

TABLE 3.—THE EFFECT OF REMOVAL OF EUGLOBULIN BY PRELIMINARY DIALYSIS ON THE ACTIVITY OF RABBIT THROMBIN.

Method.	Mg. thrombin nitrogen added to plasma.	Mg. fibrin precipitated.
Standard procedure	0.07	1.90
	0.13	2.18
	0.26	2.16
	0.66	2.45
Preliminary dialysis	0.06	0.98
	0.25	2.43
	0.69	2.91

The Preparation of Stable Dried Rabbit Thrombin. There is some evidence[9b] that rabbit thrombin in solution is reasonably stable compared with solutions of other preparations of thrombin made in this laboratory. In the course of several months the activity was found to diminish to the extent of 10%. However, for practical

reasons the preparation of a dry stable powder was necessary. Two methods were employed for this type of preparation. The final solution of rabbit thrombin was lyophilized or precipitated by acetone and the acetone precipitate dried *in vacuo*. The data presented in Table 4 indicate that when either procedure was followed there was no loss of thrombic activity.

TABLE 4.—THE EFFECT OF DRYING RABBIT THROMBIN BY LYOPHILIZATION AND ACETONE PRECIPITATION ON THE THROMBIC ACTIVITY.

Method of drying.	Mg. thrombin nitrogen added to plasma.	Mg. fibrin precipitated.
None—control solution	0.010	1.54
	0.019	1.89
	0.040	2.25
	0.100	2.51
Dried by lyophile method redissolved in 0.9% NaCl solution	0.010	1.77
	0.018	2.13
	0.040	2.39
	0.090	2.52
	0.180	2.66
Precipitated by acetone, dried *in vacuo*—redissolved in 0.9% NaCl solution	0.010	1.34
	0.014	1.88
	0.029	2.29
	0.070	2.48
	0.140	2.71

Purification of Rabbit Thrombin. Parfentjev has indicated[96] that his material may be further purified by subjecting the final solution of the pseudoglobulins to an isoelectric precipitation at pH 5.2. Therefore a preparation made by Parfentjev was compared quantitatively before and after such purification. The data are presented in Table 5. It will be observed that the isoelectric precipitate contains somewhat more thrombic activity per milligram of thrombin nitrogen than the preparation not so purified. It is debatable whether there would be any advantage in carrying through this isoelectric precipitation for material to be used in the clinic as a hemostatic. However it is of interest since it removes much of the inert nitrogenous constituents of the rabbit thrombin solution, giving a purer product for purposes of investigation.

TABLE 5.—EFFECT OF ISOELECTRIC PRECIPITATION AT PH 5.2 ON THE ACTIVITY OF RABBIT THROMBIN.

Preparation.	Mg. thrombin nitrogen added to plasma.	Mg. fibrin precipitated.
Standard procedure	0.010	1.5
	0.020	1.9
	0.040	2.3
	0.100	2.5
Isoelectric precipitation	0.003	0.6
	0.007	2.5
	0.014	2.7
	0.030	3.2
	0.040	3.2

Thermolability of Rabbit Thrombin. As will be observed from Chart 2, the solution of rabbit thrombin is thermolabile. It is less so, however, than preparations of thromboplastin. Preliminary experiments indicate that when *absolutely dry* powdered rabbit thrombin is heated in sealed containers to temperatures of 110° C. for purposes of sterilization, there is little loss of potency. However, the presence of even small amounts of water in the material results in the destruction of the activity of the thrombin when it is exposed to these high temperatures.

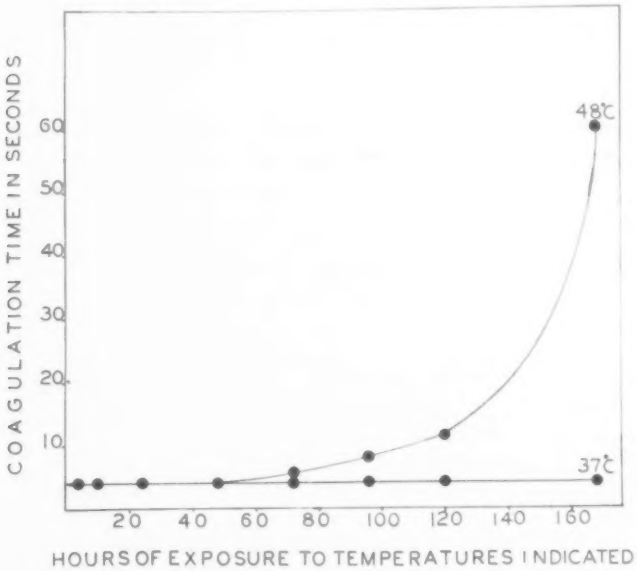

CHART 2.—The effect of temperature on the potency of rabbit thrombin. (0.1 ml. of a 1 to 10 dilution of rabbit thrombin added to 0.1 ml. of normal human plasma. Each test repeated at the time intervals shown.)

A Comparison of Rabbit Thrombin With Thrombins of Other Origin. In order to compare rabbit thrombin and thrombin prepared by other methods with regard to their action on fibrinogen of plasma, a series of quantitative experiments were performed. Thrombin prepared by the method of Seegers *et al.*[11] and by the conversion of prothrombin by rabbit brain thromboplastin in the presence of calcium were used in these studies. The data obtained on these studies are presented in Table 6. It will be observed that the action of rabbit thrombin is entirely comparable to that of thrombin prepared by these other methods.

Discussion. Thrombin by definition is a substance or a physiological activity, capable of directly converting fibrinogen to fibrin without the necessity of calling into operation calcium, prothrombin

and thromboplastin. It is therefore, to say the least, surprising that one may obtain directly from citrated rabbit plasma a substance possessing true thrombic activity. The observations recorded in this communication indicate quite clearly that when rabbit plasma is treated with certain salting-out procedures a pseudoglobulin may be obtained which does indeed possess thrombic activity. However, it must be remembered that at present we can offer no evidence that this thrombic activity is not due to changes in plasma proteins produced by manipulation involved in the procedure.

TABLE 6.—A COMPARISON OF RABBIT THROMBIN PREPARED BY PARFENTJEV'S METHOD WITH THROMBIN PREPARED BY THE DIRECT CONVERSION OF PROTHROMBIN OF STEER BLOOD, BY THROMBOPLASTIN IN THE PRESENCE OF CALCIUM.

Preparation.	Method.	Mg. thrombin nitrogen added to plasma.	Mg. fibrin precipitated.
Seegers *et al.*[11]	Prothrombin of steer blood + steer lung thromboplastin	0.0600	0.45
		0.1900	1.51
		0.3100	4.05
Seegers *et al.*[11]	Prothrombin of steer blood + steer lung thromboplastin	0.0015	2.42
		0.0300	4.34
		0.0900	4.76
Lozner, Adams and Taylor (unpublished)	Prothrombin of steer blood + rabbit brain thromboplastin	0.0005	2.10
		0.0009	2.60
		0.0018	4.08
		0.0090	5.54
Lozner, Adams and Taylor (unpublished)	Steer globulin substance + rabbit brain thromboplastin	0.0008	1.64
		0.0010	1.57
		0.0060	1.63
		0.0170	2.44
Parfentjev	Pseudoglobulin of rabbit plasma	0.0100	1.54
		0.0200	1.89
		0.0400	2.25
		0.1000	2.51
Parfentjev	Pseudoglobulin of rabbit plasma isoelectric precipitation	0.0030	0.58
		0.0070	2.46
		0.0100	2.72
		0.0300	3.18
		0.0400	3.24

Should thrombin exist preformed in rabbit plasma *in vivo*, obviously the presence in rabbit plasma of a substance capable of neutralizing it *in vivo* must be predicated. To account for the possible occurrence of thrombin in rabbit plasma several facts may be considered. It is conceivable that slow conversion of prothrombin to thrombin occurs. This is suggested indirectly both by the rapid disappearance of prothrombin from citrated rabbit, human and steer plasma and the frequent observation that small amounts of fibrin separate from citrated or oxalated plasma on standing. This phenomenon has been commented on by other authors.[2,8] It is also of importance to consider the species of animal plasma involved in the present observations. It is well known that rabbit blood has a remarkably short coagulation time. It is also noteworthy that the thromboplastic activity of rabbit brain extract is much greater than that of similar extracts from steer, sheep or human brains.[12]

That this enhanced thromboplastic titer is shared by other rabbit tissues is a distinct probability. Should rabbit plasma have an increased thromboplastic titer, the conversion of prothrombin to thrombin would probably be accelerated.

There is some evidence of a preliminary nature[1] that while the potency of rabbit thrombin is not increased by the age of the plasma the total yield of thrombin appears to be increased.

The euglobulin fraction of plasmas of various species of animals has been shown to contain clot-promoting activity for hemophilic blood.[6,10] We have called this clot-promoting substance or activity "globulin substance,"[10] and Howell has called it "plasma thromboplastin."[5] The data presented in this paper indicate that after this euglobulin fraction was removed from rabbit plasma by dialysis for 8 days, the supernatant fluid when treated by Parfentjev's procedure yielded a pseudoglobulin having unimpaired thrombic activity. This observation seems to differentiate "globulin substance" or "plasma thromboplastin" from rabbit thrombin, however here also one must reserve the possibility of protein change due to the subsequent salting-out procedure. Further differentiation is seen in the fact that the euglobulin fraction containing the thromboplastin factor requires both calcium and prothrombin to be present before fibrinogen can be precipitated from normal plasma. The pseudoglobulin fraction containing rabbit thrombin on the other hand can act on fibrinogen directly, in plasmas containing an excess of citrate or oxalate and also on plasmas from which the prothrombin has been removed by adsorption.

The amount of calcium present in rabbit thrombin is too small to initiate blood coagulation in the presence of citrate or oxalate. However, we have not as yet been able to prepare by any method a thrombic substance which does not contain this small amount of calcium which is apparently closely associated with the protein of the preparation.[1]

Solutions of rabbit thrombin in sterile form are quite stable, compared with solutions of thrombin prepared by other methods. Some of the preparations used in these studies were 7 months old and still possessed marked activity. The thermolability of rabbit thrombin is fairly low. Absolutely dry rabbit thrombin may be sterilized in sealed containers at 110° C. The low thermolability also permits the drying under reduced pressure of gauze and similar material impregnated with solutions of rabbit globulin.

A completely stable dry powdered preparation may be obtained by the method of vacuum drying at low temperatures such as is used in the lyophile process or by precipitation of the pseudoglobulin from its final solution with acetone and subsequent drying *in vacuo*. Both preparations are of equal activity, and show no loss of potency as compared to the original pseudoglobulin solution from which they were derived. The high potency, ease of prepara-

tion and high yield of rabbit thrombin would clearly indicate the desirability of clinical trial of the material as a hemostatic. Certain observations concerning the use of rabbit thrombin in the control of hemorrhage are presented in the following paper.

Conclusions. 1. The observation of Parfentjev that rabbit plasma contains a substance or physiologic activity capable of converting fibrinogen to fibrin is confirmed.

2. Parfentjev's material is apparently a pseudoglobulin which possesses true thrombic activity.

3. It may be differentiated from thromboplastin by its manner of preparation and its independence of calcium-ion concentration in its action on fibrinogen.

4. Methods of preparing the material in a stable powdered form are presented.

A material has been prepared from the plasma of steer by the procedure outlined in this paper possessing similar properties to that obtained from rabbit plasma.

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THE USE OF RABBIT THROMBIN AS A LOCAL HEMOSTATIC.

By Eugene L. Lozner, M.D.,

assistant in medicine, harvard medical school; resident physician, thorndike memorial laboratory, boston city hospital,

Harriet MacDonald, B.S.,

laboratory assistant, thorndike memorial laboratory, boston city hospital,

Maxwell Finland, M.D.,

assistant professor of medicine, harvard medical school; assistant physician, thorndike memorial laboratory; junior visiting physician and chief, fourth medical service, boston city hospital,

and

F. H. L. Taylor, Ph.D.,

research associate in medicine, harvard medical school; chemist, thorndike memorial laboratory, boston city hospital,

boston, mass.

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.)

The body possesses two main defenses against hemorrhage, the coagulation of the shed blood and the mechanical behavior of blood vessels toward injury. Thrombin precipitates fibrin instantaneously,

independently of the other factors in the blood coagulation reaction, and thus can be of value in aiding local hemostasis. It has been used therapeutically in this manner with encouraging results.[4,6] The preparation of this physiologic complex however has in the past been difficult and time-consuming and only small yields were obtained from relatively large amounts of plasma. Increased availability has been achieved because of the isolation by Parfentjev, from rabbit plasma, of a globulin fraction able to clot citrated blood.[2] This fraction has been identified by this laboratory as possessing great thrombic activity. The details of this identification are presented in the preceding paper.[5] Translating the results into practical terms, one part of dry powdered rabbit thrombin will clot 60,000 parts of 0.25% citrated blood within three seconds. The potency of the material, the shortness of the time necessary for *in vitro* clotting, the simplicity of preparation and large yields, made it desirable that the hemostatic properties of rabbit thrombin be investigated clinically. This communication reports the results of these investigations.

Methods. Three types of preparations of rabbit thrombin were used, a saline solution containing from 10 to 12% total solids, a dry sterile powder prepared by lyophilization and a dry sterile powder prepared by acetone precipitation and subsequent *in vacuo* desiccation. Some of the materials used were prepared in this laboratory, others were furnished by Dr. Parfentjev. With these preparations, two types of investigations were undertaken; a controlled study of hemostasis following a standard trauma in normal subjects and observations on the use of rabbit thrombin in the control of bleeding from small wounds in patients with and without hemorrhagic diseases.

Controlled Study of Hemostasis in Normal Subjects. A standard trauma was utilized in order to study hemostasis in the normal subject. For this purpose a modification of the Ivy bleeding time[1] was used. A sphygmomanometer cuff was placed around the upper arm of the subject and inflated to 50 mm. Hg pressure. A wound with an ordinary mechanical lancet was then made on the ulnar side of the flexor surface of the forearm, avoiding visible veins, approximately 3 mm. in width and 4 mm. in depth which produced bleeding lasting from 5 to 7 minutes when not treated. The amount of bleeding was measured by gently approximating a square of filter paper to the drop of blood over the wound at 30-second intervals. When the bleeding stopped, the time was recorded and the areas of the drops of blood on the filter papers were measured and totalled. Four methods of treatment with rabbit thrombin were investigated. Controls were repeated in identical fashion except for the omission of rabbit thrombin.

The details of the four methods of treatment are as follows:

I. "*Wet Spray:*" The wound was allowed to bleed for 60 seconds and was then sprayed with the solution of rabbit thrombin for 15 seconds using a "nebulizer." Following this procedure the amount of bleeding was determined as described above.

II. "*Dry Spray:*" Dry powdered rabbit thrombin was applied to the wound in identical fashion to that described for the solution.

III. "*Wet Application:*" Immediately following the trauma a square of filter paper moist with the solution of rabbit thrombin was applied to the wound. This was kept in apposition to the wound without pressure for

2 minutes. The amount of bleeding following this was measured and added to the amount of bleeding on the filter paper used in the treatment. For the controls to these observations a square of filter paper moist with saline was applied in an identical fashion.

IV. "*Dry Application:*" This procedure was identical with that used for the application of the solution of rabbit thrombin except that the square of filter paper applied to the wound was dampened with saline and sprinkled with a small amount of dry rabbit thrombin.

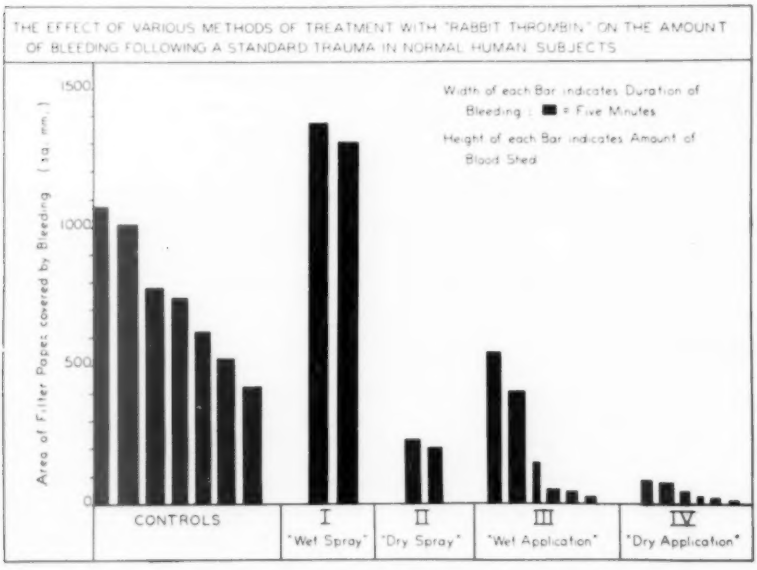
THE EFFECT OF VARIOUS METHODS OF TREATMENT WITH "RABBIT THROMBIN" ON THE AMOUNT OF BLEEDING FOLLOWING A STANDARD TRAUMA IN NORMAL HUMAN SUBJECTS
Area of Filter Paper covered by Bleeding (sq. mm.)
1500
1000
500
0
Width of each Bar indicates Duration of Bleeding: ■ = Five Minutes
Height of each Bar indicates Amount of Blood Shed
CONTROLS
I
"Wet Spray"
II
"Dry Spray"
III
"Wet Application"
IV
"Dry Application"

CHART 1.

Results. The results of this study are presented in graphic form in Chart 1. It will be observed that all methods of treatment except that of the "Wet Spray" considerably decreased the amount of bleeding. The most effective method was that of "Dry Application." In general, the dry powdered rabbit thrombin gave better results than the solution. The apparent prolongation of bleeding produced by the "Wet Spray" was the result of the instantaneous formation of a flimsy clot which, however, did not adhere to the wound and beneath which the bleeding continued.

Observations on the Use of Rabbit Thrombin in the Control of Bleeding in Patients. Observations were carried out on 11 patients who were bleeding from *small wounds*. Nine of these had hemorrhagic diatheses, 4 with hemophilia, 3 with symptomatic thrombocytopenic purpura, 1 with hypoprothrombinemia and 1 with hereditary thrombasthenia. The other 2 patients had hypertension with epistaxis. Dry powdered rabbit thrombin was usually used and the technique of application was in general comparable with that pre-

viously described.[3] Essentially this consists of impregnating with the dry powder a dressing, pack or "gauze bite" which has been dampened with sterile saline. This impregnation is carried out in such a fashion as to allow the dry powder to come into immediate contact with the bleeding surface.

TABLE 1.—SUMMARY OF OBSERVATIONS ON THE USE OF RABBIT THROMBIN AS A LOCAL HEMOSTATIC IN 13 PATIENTS.

Pt. No.	Obs. No.	Age (yrs.)	Diagnosis.	Bleeding site.	Method of treatment.	Duration of hemorrhage prior to institution of rabbit thrombin treatment (days).	Degree of hemostasis.
1	1	28	Hemophilia	2.5 cm. laceration on hand	10% solution on dressing	4	Complete
2	2	36	Hemophilia	0.6 cm. laceration in antecubital vein	Powder on dressing	*	Complete
3	3	7	Symptomatic thrombocytopenic purpura (acute leukemia)	Sternal biopsy	Powder on dressing	1	Complete
4	4	23	Hemophilia	Gum	Powder on "gauze bite"	5	Complete
5	5	22	Hemophilia	Tooth socket	Powder on "gauze bite"	*	Complete†
5	6	22	Hemophilia	2 tooth sockets	Powder on "gauze bite"	*	Complete‡
6	7	24	Symptomatic thrombocytopenic purpura (aplastic anemia)	Stab wound in hemarthrosis	Powder on dressing	2	Complete
7	8	46	Symptomatic thrombocytopenic purpura (aplastic anemia)	Nose	Powder on pack	1	Partial once Complete twice
8	9	41	Hypertension	Nose	Powder on pack	14	Complete
9	10	52	Hypertension	Nose	Powder on pack	5	Complete
10	11	48	Hypoprothrombinemia (non-tropical sprue)	Nose	Powder on pack	1	Complete
11	12	33	Hereditary thrombasthenia	Tooth socket	Powder on "gauze bite"	2	Complete
2	13	36	Hemophilia	Tooth socket	Powder on "gauze bite"	*	Complete§

* In these patients rabbit thrombin was used immediately following the trauma.
† "Bite" changed every 24 hours, socket healed in 8 days.
‡ "Bite" changed every 24 hours, socket healed in 7 days.
§ "Bite" changed every 24 hours, socket healed in 4 days.

For purposes of brevity the clinical observations have been summarized in Table 1. With only one exception (Patient 8), the details of which are presented below, the hemostasis produced by rabbit thrombin was complete and immediate. That is to say, as soon as the dressing, pack, or "gauze bite" containing rabbit thrombin was applied to the bleeding surface, arrest of hemorrhage immediately occurred. Prior to the application of rabbit thrombin in these patients, hemorrhage had occurred for varying lengths of time. In 9 of the 11 patients this hemorrhage had not been controlled by various hemostatics, including pressure bandaging, and application or tamponage with adrenalin, ferric subsulphate solution and animal muscle. In one (Patient 9) with hypertension and epistaxis, bleeding had persisted for 14 days prior to rabbit thrombin

therapy despite all the above measures and four transfusions of blood.

In Patient 8, mentioned above, in which hemostasis on the first application of rabbit thrombin was only partial, the diagnosis was symptomatic thrombocytopenic purpura associated with aplastic anemia. Two subsequent applications on following days produced complete and immediate hemostasis. The unsatisfactory result on the first application was probably due to the failure of the operator to bring the rabbit thrombin into immediate contact with the bleeding site.

The dry powder was employed in the majority of patients exclusively. However, isolated observations carried out with rabbit thrombin solution indicated that it, too, was of value as a local hemostatic, but perhaps not quite as effective as the dry powder. No toxic effects from the application of rabbit thrombin were observed in any of the normal subjects or patients, no matter in what form it was applied.

Discussion. The above observations indicate that rabbit thrombin is of distinct value as a local hemostatic in small wounds. This apparently results from the property of this material very rapidly to precipitate fibrin and thus close the wound.

Numerous practical methods for the application of rabbit thrombin suggest themselves. Such methods, however, undoubtedly will depend on the location of the bleeding site and the personal choice of the surgeon. Gauze bandages impregnated with thrombin would seem to be likely of success in controlling bleeding from wounds on the extremities. It is important that the particular method employed be one in which the highest concentration of rabbit thrombin comes into contact with the bleeding surfaces. In many situations this is more feasible with the dry powder than with a solution.

It should not be forgotten that in addition to the factor of coagulation of blood when shed, the factor of vascular defense is important in determining the duration of bleeding. Certain situations occur where the blood vessel factor fails to such an extent that no hemostatic which depends for its action on the formation of fibrin can be effective.

It must also be borne in mind that the parenteral administration of thrombin is very toxic and may result in generalized thrombosis. It is important therefore that the material be used cautiously.

Summary and Conclusions. 1. Observations are reported indicating that rabbit thrombin is of distinct value in the control of hemorrhage from *small wounds* induced by a standard trauma in normal persons and in 9 patients with and 2 without hemorrhagic diatheses bleeding spontaneously from small wounds.

2. The material apparently accomplishes its results by the instantaneous precipitation of fibrin.

3. No toxic manifestations were observed following the application of rabbit thrombin to the small wounds.

4. It would appear desirable that these observations concerning the use of rabbit thrombin as a local hemostatic be extended.

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BOOKS RECEIVED.

NEW BOOKS.

Theory of Occupational Therapy for Students and Nurses. By NORAH A. HAWORTH, M.A. (CANTAB.), M.R.C.S., L.R.C.P., D.P.M., Late Senior Assistant Medical Officer, Severalls Mental Hospital, Colchester, and Honorary Assistant Physician, Lady Chichester Hospital for Functional Nervous Diseases, Hove; and E. MARY MACDONALD, Principal, Dorset House School of Occupational Therapy, and Occupational Therapist in charge of the Allendale Curative Workshop, Bristol. With a Foreword by SIR ROBERT STANTON WOODS, M.D., F.R.C.P., Consultant Adviser in Physical Medicine to the Ministry of Health, etc. Pp. 132; 81 illustrations. Baltimore: The Williams & Wilkins Company, 1941. Price, $2.00.

The Intervertebral Disc. With Special Reference to Rupture of the Annulus Fibrosus With Herniation of the Nucleus Pulposus. By F. KEITH BRADFORD, M.D., Houston, Texas, and R. GLEN SPURLING, M.D., Louisville, Ky. Pp. 158; 45 illustrations. Springfield, Ill.: Charles C Thomas, 1941. Price, $4.00.

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PROGRESS
OF
MEDICAL SCIENCE

OTO-RHINO-LARYNGOLOGY

UNDER THE CHARGE OF
JOSEPH C. BECK, M.D.,
ASSOCIATE PROFESSOR EMERITUS, DEPARTMENT OF OTO-LARYNGOLOGY, UNIVERSITY OF ILLINOIS COLLEGE OF MEDICINE,
AND
NOAH D. FABRICANT, M.D.,
ASSOCIATE, DEPARTMENT OF OTO-LARYNGOLOGY, UNIVERSITY OF ILLINOIS COLLEGE OF MEDICINE, CHICAGO, ILLINOIS.

NASAL PHYSIOLOGY.

It is only within recent years that the interest of rhinologists has been drawn to the much neglected field of nasal physiology. Previously rhinologists centered their energies on anatomy, surgical anatomy—and more recently— on pathologic anatomy. The result has been a period of satisfactory growth. With the recognition of the importance of the functional point of view in rhinology, it is quite conceivable that nsaal physiology will bloom into deserving maturity. The importance of knowing how a nose works is stressed by Proetz,[10] who deplores the small amount of space given in standard textbooks and monographs to nasal physiology. There are many pitfalls that may beset the rhinologic surgeon unless he studies the functional viewpoint. For old methods, old drugs and old operations should be abandoned when they have been discredited.

Frenckner and Richtner[4] report a study of ciliary movements in living and anatomic sections under normal and pathologic conditions. The beating frequency of the cilia was found to vary between 160 and 250 beats per minute under normal conditions. Repeated infections of the nasopharynx seemed to arrest ciliary activity. Ethyl chloride had a certain resting effect, but ether narcosis and injection narcosis with pernocton and urethane seemed to be innocuous. Certain local anesthetics checked the ciliary activity, at least temporarily. Experiments on animals appear to show that a long hypovitaminosis with regard to vitamins A, C and D causes slowing of the ciliary activity but that a short, complete avitaminosis does not have any obvious effect. No change in ciliary activity was caused by administration of hormones. Anatomic sections immersed in Ringer's solution showed no inhibition of ciliary activity for a period of 6 to 10 hours. In physiologic salt

solution the frequency of the ciliary beats was visibly lessened somewhat earlier. Weak solutions of ephedrine had a slightly stimulating effect. At no time was complete inhibition observed. Dilute solutions of adrenalin had an inhibitory effect on mucous membrane sections, but the ordinary 1–1000 solution had no visible effect on mucous membrane of living animals. Weak colloidal silver preparations appeared innocuous, while ephedrine solution combined with 5% neoprontosil solution had a stimulating effect. Prontosil injected into living guinea-pigs produced no visible change in ciliary activity. As a general rule, expectorants and volatile oils, applied locally or in the form of vapor, produced slowing of ciliary activity following a brief period of stimulation. The inhibitory effect produced by many of the agents was found to be less pronounced in the mucous membrane of a living person than in extirpated mucous membrane sections. Leasure[7] emphasizes the importance of the thin sheet of mucus which covers the mucous membrane of the respiratory tract. The mucous membrane of the respiratory tract, especially that of the nasal portion, needs active cilia moving a sheet of mucus in order to be healthy. At times perfectly healthy nasal mucous membrane is quite capable of resisting the most virulent invader. Any inhibition of the production of mucus constitutes an exposure. Secretory parasympathetic innervation to the nasal mucous glands has been reported. A predominant activity of the sympathetic nervous system is found to coincide with circumstances associated with common exposures to cold.

In their study of the effect of estrogens on ciliated mucosa, Boyd and his associates[1] reveal that cilia of the bucco-esophageal mucosa of the frog are most sensitive to estradiol and least sensitive to estrone and that the sensitivity to estriol lies midway between. In minute amounts, too small to affect the hydrogen-ion concentration or the electrolyte concentration, these estrogens first stimulate and then, in larger doses, depress ciliary movement. If the value of 1 is given to the stimulating power of estrone toward ciliary movement, then estriol may be calculated to be twenty times as active as estrone and estradiol one hundred times as active. This order of activity toward cilia does not correspond to the order of estrogenic activity of these crystalline substances. If estrone is given an estrogenic activity of 1, estriol may be estimated as one one-hundredth as active and estradiol as ten times as active as estrone. Since cilia form an integral part of the protoplasm of the mucosal cells, the order of activity of estrogens on ciliary movement may reflect a corresponding order of activity on other functions of mucosal protoplasm. Among these other activities, the authors were especially interested in the power of estrogens to relieve ozena and atrophic rhinitis, which has been reported by other investigators. If the present data have any application to the latter studies, they indicate that dosage is an extremely important factor. Nash[8] declares that the function of a mucous membrane is to produce mucus, but the nasal mucous membrane must produce mucus within certain quantitative limits if the nasal functions are to be maintained perfectly. Any shifting of this mucus production into either a hypofunction or a hyperfunction will result in a functional nasal disturbance, and the signs and symptoms of this disturbance will depend upon the amount and character of the dysfunction and the length of time it has continued.

A normally functioning nasal mucous membrane produces a limited amount of mucus. It does not hypofunction nor does it hyperfunction. Hypofunctional diseases, such as atropine rhinitis, physical rhinitis, psychic rhinitis, atrophic rhinitis, febrile rhinitis and debilitative rhinitis, begin with dryness of the mucous membrane, and if long continued terminate in atrophy. Hyperfunctioning diseases, such as irritative rhinitis, infective rhinitis, allergic rhinitis and the rhinitides produced physically, psychically and by food and the endocrines, begin with engorgement and rhinorrhea, and if long continued terminate in hyperplasia. Relatively few agents or conditions produce hypofunctioning diseases, but many produce hyperfunctioning diseases.

Fabricant,[3a–c] in receiving the Casselberry Prize Award of the American Laryngological Association for 1941, stressed the significance of the pH factor as a basis for the treatment of upper respiratory infection. With the advent of comparatively new and simple methods for measuring hydrogen-ion concentration, it is possible to awaken the clinician's interest in a type of investigation that has practical application to innumerable and diversified fields. To offset the fact that nasal secretions, when collected, are susceptible to changes in pH almost instantly when they are exposed to air and are divorced from the normal milieu of the nose, the use of the glass electrode with its associated measuring equipment, the Coleman electrometer, has made it feasible to measure the pH of nasal secretions *in situ* with ease and precision. By conducting minute-to-minute readings of the pH values of nasal secretions in a group of patients observed daily for 2 years and graphically charting the results, it has been possible to explain adequately the wide range of nasal pH revealed in contributions elsewhere by workers employing other methods of procedure in studies of clinically normal nasal conditions and of acute rhinitis, acute rhinosinusitis and allergic rhinitis. The pH of nasal secretions *in situ* describes a series of high and low points. At no time does the pH remain constant over an appreciable period. Its fluctuations in a clinically normal nose—from approximately 5.5 to 6.5—appear to follow a definite course. The futility of relying on only one pH reading is amply illustrated by the frequent numerical changes in pH values that occur when readings are recorded over one hour or more. The course of nasal pH is altered by the application of either cold or heat. Cold produces a drift toward alkalinity, heat a drift toward acidity. The pH of nasal secretions *in situ* during an attack of acute rhinitis or acute rhinosinusitis or during the more active phases of an attack of allergic rhinitis is alkaline. This finding has clinical significance, for those nasal vasoconstrictors which can lower the nasal pH from an abnormal alkaline status to a normal slightly acid status—a pH level between approximately 5.5 and 6.5—may perform a valuable function. Conversely, those nasal vasoconstrictors which raise, enhance or perpetuate an alkaline nasal pH during these rhinologic conditions may prolong an undesirable status of nasal pH. Acceptable nasal therapy for acute rhinitis and acute rhino-sinusitis often includes the careful selection of an appropriate nasal vasoconstrictor, counseling the patient to obtain adequate rest and sleep, and the judicious administration of external heat for the relief of pain in selected cases of acute nasal sinusitis. Each of these well-recognized therapeutic measures

produces but a single, uniform nasal pH phenomenon—acidity. All elective nasal surgery procedures which require the use of topical anesthetics and vasoconstrictors are performed in a completely acid nasal pH environment. At this time the pH of the nasal secretions *in situ* is acid, the topical anesthetics themselves are acid, the vasoconstrictors are acid, and the noticeable effect of the topical anesthetics, alone or in combination with a few drops of a vasoconstrictor, is acid. The actual return of the nasal pH to an acid status is accomplished—unwittingly or no—by every physician who prescribes certain well recognized nasal vasoconstrictors. Should the occasional therapeutic nihilist omit the use of a nasal vasoconstrictor but, in turn, utilize such measures as adequate sleep and rest and external heat, singly or collectively, the end result can be only that of establishing an acid nasal pH. Fabricant suggests that every nasal vasoconstrictor available to the public should satisfy two basic physiologic requirements: 1, the restoration and maintenance of normal ciliary activity; and 2, the possession of a slightly acid pH value between 5.5 and 6.5. He also suggests that silver preparations be standardized to a similar level, for those silver preparations, nasal vasoconstrictors and antiseptics which either raise, enhance or perpetuate the alkaline nasal pH found in acute rhinitis and acute rhinosinusitis prolong an undesirable status of nasal pH—an alkaline status in which the bacteria accompanying the acute infection find a fertile field for growth. To divorce and separate the ciliary factor from the pH factor is to tell but half the story of applied nasal physiology.

Physiologic experiments prove that an inspiratory stream of air can be deflected and broken by the shape and position of the nasal aperture and also by any mechanical obstacle or alteration in the lateral and medial walls of the nose. The various structures which, if abnormal, can deflect these inspiratory air currents may be grouped under the general term "nares." According to Cinelli,[2a,b] they are the columella and anterior portion of the nasal septum, the alar cartilages with lateral and medial crura, the upper lateral cartilages and the small constrictor-dilator muscles of the nose. Collapse of the nares may be either functional or organic. In the functional type, the structural tissue of the nose is normal, but the patient does not breathe correctly. "He breathes with the nose and not through the nose." The proper corrective measure is to have the patient, before a mirror, breathe in slowly, deeply and long, with the nares intentionally dilated outward. The organic type of collapse of the nares may be incomplete or complete. The incomplete collapse is due to a weakened or atrophic condition of the upper lateral cartilage. Correction is obtained by the implantation of cartilage with its attached outward perichondrium, which strengthens the middle third of the lateral wall, thereby giving sufficient mechanical support to prevent it from impinging toward the septum. The complete organic collapse is due not only to atrophy of the upper lateral and alar cartilages but chiefly to atrophy of the dilator and constrictor muscles of the nares. The nasal muscles play an important part in the physiology of the nose and in the physiognomy, character and personality; they are a source of emotional expression in the daily tasks and an understanding of their nature and function is of importance to persons engaged in the arts of painting and sculpture. To consider these muscles rudimentary would be to destroy man's weapon of individuality.

The correction of complete organic collapse of the nares is surgical. The nares are transfixed laterally in such a position that they cannot collapse. The result is immediate improvement in breathing.

The surgical establishment of nasal ventilation is discussed by Parkinson.[9] He writes that inadequate nasal ventilation is a common and important problem. It is due to a variety of pathologic entities, the most usual being persistent congestion, persistent edema, deviated septum and mucosal hyperplasia. Many cases are relieved by allergic or endocrine control, or by nasal or sinal treatment. The remaining causes can be relieved by intranasal surgery following sound and approved principles. As the causes of chronic obstruction vary, surgical measures must vary accordingly. Besides submucous resection, measures are outlined for resetting the inferior turbinate, reducing its erectile capacity, and removing portions that are hyperplastic. Intranasal antral windows are useful as additional space for resetting the inferior turbinate. This is recommended as a vlauable addition to ventilation surgery. Improved ventilation brings improvement in nasal physiology. Hilding[5] finds that rabbits develop suppurative sinusitis readily as a result of operative windows made between the maxillary sinus and the nasal cavity. Sinusitis is much more apt to occur if the window is made at or near the ostium than if it is made at a point distant from the ostium. A sinus may not be mutilated surgically with impunity in disregard of the physiology of the ciliary mechanism. Apparently, open windows, like scars, may disrupt sinus drainage if placed in positions where they interfere with ciliary flow. It is possible to make windows in such positions with reference to the ciliary mechanism that they do not seriously interfere with drainage. In a thoughtful discussion of the relationship of nasal obstruction to impairment of hearing, Johnson[6] states that the presence of an anatomic obstruction in the nose cannot affect the eustachian tube either by misdirecting the air currents in the nasopharynx or by causing inflammatory reactions in the tube. If, on the other hand, this obstruction causes a prolongation of infections of the upper respiratory tract, so that they more frequently go on to sinusitis, it is likely that the presence of infections in the nose and in the nasopharynx would cause inflammatory reactions in the tube that might lead to adhesive processes in the middle ear. The inflammatory change, therefore, has been the result of the infection and not the direct result of the obstruction. The author reviews 46 cases of submucous resection for nasal obstruction. In 65% (30 cases) there was either no deficiency in hearing or only high tonal dips. In the cases in which some improvement of hearing was present the audiometric improvement was not sufficient to warrant urging the operation as a means of improving hearing.

NOAH D. FABRICANT, M.D.

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DERMATOLOGY AND SYPHILOLOGY.

UNDER THE CHARGE OF

JOHN H. STOKES, M.D.,

HERMAN BEERMAN, M.D., AND NORMAN R. INGRAHAM, JR., M.D.,

PROFESSOR AND ASSISTANT PROFESSORS, RESPECTIVELY, DEPARTMENT OF DERMATOLOGY AND SYPHILOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA.

THE TRIVALENT ARSENICALS IN SYPHILIS.

PART II. TREATMENT REACTIONS.

THIS review is a continuation of the discussion of the trivalent arsenicals in syphilis which we began in a previous issue of this Journal (AM. J. MED. SCI., **201**, 611, 1941).

It is hardly necessary in a modern discussion of reaction to treatment with the antisyphilitic drugs to stress the importance of these reactions to the future of the syphilis program. It is obvious that the practising physician is ever seeking an arsenical preparation which will be both easy to administer, and when administered least incommoding to the patient, and at the same time, will produce the desired therapeutic effect. Although progress in this direction has been made recently (arsenoxide—Mapharsen), previous discussion has pointed out that the ideal has not yet been attained. Accordingly, it is still necessary for the physician to have a clear understanding of the types and significance of the various untoward reactions which he may encounter in the management of his patient with syphilis. It is not feasible in so short a review to give all the clinical data necessary for recognition of the various types of reaction. An attempt will be made, however, to analyze the various factors which may play a part in the production of these by-effects or arsenical medication. For purposes of discussion we have followed the classification of treatment reactions to the arsenicals, proposed by the Coöperative Clinical Group[31a] as follows:

I. *Mild.*
- A. Gastro-intestinal reaction
- B. Nitritoid reaction
- C. Pruritus
- D. Slight skin eruptions
- E. Transient kidney irritability

II. *Severe.*
- A. Acute yellow atrophy of the liver
- B. Aplastic anemia
- C. Arsenical stomatitis
- D. Crustaceous dermatitis
- E. Icterus
- F. Ocular damage
- G. Purpura hæmorrhagica
- H. Venous embolus
- J. Death

This classification is not all-inclusive, but is sufficient for practical purposes.

Incidence. Relatively few large-scale or intensive studies of reaction to arsenicals have appeared in recent years. Waugh and Milovich[179] have recently reviewed severe reactions to arsphenamine; various papers on treatment reactions in the United States Navy have been collected by Lt. Com. Stephenson and his co-workers;[33,149–151,152a,b] and reports by Ervin Epstein[41d] on cutaneous reaction to Mapharsen; by Ireland;[73] the collected reviews by Cole and his associates,[30] and the Coöperative Clinical Group studies[31a] on arsenical reactions are the principal American contributions. In evaluating published accounts of various reactions to the arsenicals, the degree of particularity with which the observer has collected his material must be considered. Certain individuals will record the slightest reaction as significant, and thereby include in their totals data which do not figure in the estimates of other observers. Others rate only serious reactions as worthy of statistical estimation. The mechanical processes of collecting and recording reactions to treatment with the arsenicals affect the statistical results. It follows that two observers equally competent, working in presumably equally ideal situations, come to widely differing conclusions from their data. The following account summarizes some of the factors that appear to affect the occurrence of reaction in the treatment of syphilis with the arsenicals.

Age, Sex, Race. The majority of reactions occur in the age groups in which intolerance of the drugs due to functional impairment of advancing years is to be expected. A partition of reaction incidence between youth and age was observed by the Coöperative Clinical Group[31a] which found that there was an increased incidence of mild reaction in the age groups 15 to 19 and 20 to 24; consisting especially of gastro-intestinal and nitritoid reactions. As the age of the patient increased, mild reactions showed a tendency to decrease, but severe reactions tended to increase in frequency. This was especially true for crustaceous dermatitis (except in the 15 to 19 age group) and icterus, which rose from an incidence of 0.5% in the age group 15 to 19 to 1.6% in the age group 45 to 49. Classifying reactivity by type of syphilitic involvement present confirmed these findings. Cole and his associates[30] found that in older individuals treated with arsphenamine, more icterus was seen than among younger patients. Skin eruptive reactions were more frequent below 35; pruritus above 35. Nitritoid reactions were observed more frequently after this age. In contrast with the earlier Coöperative Clinical Group findings, severe crustaceous dermatitis was observed almost entirely before the age of 35. Hemorrhagic encephalitis was found by Cole and his associates[30] to occur more frequently in young adulthood. The Coöperative Clinical Group found that mild arsenical reactions were twice as frequent in females as in males in both colored and white races; while severe reactions were seen more frequently in whites without regard to sex. Cole and coworkers showed, furthermore, that white women are more reactive than negro women. Waugh and Milovich,[179] reporting the experience of the Hot Springs treatment center of the United States Public Health Service, found that there were 2.47 severe reactions per 1000 arsenical injections among the men, and 2.39 among the women. The incidence

was higher for white men than colored men, but was higher for colored women than for white women. The single case of acute yellow atrophy of the liver observed at Hot Springs occurred in a white man, and the single case of agranulocytopenia with angina occurred in a white woman with secondary syphilis. These observers found 1.83 icterus reactions per 1000 arsenical injections among the men, and 1.14 among the women; and no difference in the incidence of icterus as between white and colored patients. Crustaceous exfoliative dermatitis was more than twice as common among women as among men; and about the same for the white and colored races. They found, furthermore, that this reaction was almost twice as common among white women as among white men, and four times more common among colored women than colored men. While the United States Naval statistics[33,149,151,152a,b] cannot be used for comparative purposes here, the generally accepted idea that women are more reactive than men may to some extent account for the low incidence of reaction in the Naval statistics which concern men, and young men at that. The rôle of pregnancy and other factors explaining the apparently increased incidence of treatment reactions to the arsenical in women will be presently discussed.

Rajam[122] reported his experience with reactions and complications in the course of administration of 24,550 doses of neoarsphenamine to 7160 East Indians with syphilis. His statement that this race does not tolerate neoarsphenamine is well borne out by his figures; dermatitis was produced in 1 of each 126 patients treated, an incidence of 1 to 147 for men and 1 to 83 for women. The individual dose of neoarsphenamine never exceeded 0.3 gm., yet there was 1 case of dermatitis for each 431 injections; 1 to 514 for the men and 1 to 264 for the women. There were 3 deaths. Jaundice, however, was less than half as common, but in this series hemorrhagic encephalitis developed in 6 cases. In his masterly study of blood dyscrasias in the treatment of venereal diseases, the late E. T. Burke[18] found that approximately 0.5% of women patients and 0.3% of male patients in his Whitechapel series (1934–38) developed blood dyscrasias. Burke found sulfarsphenamine to be the common factor in all of his cases, but does not state whether more men or more women were treated with this drug.

In connection with the effect of race, Sampson and Latven[136] showed that there is a definite and constant difference in the tolerance to neoarsphenamine of albino rats from different colonies or racial strains. It is apparent from their experiments that it is not possible to make a significant comparison of toxicity among samples of neoarsphenamine when rats from different strains are used.

Relation of Number of Treatments and of Dosage to the Frequency of Arsenical Complications. There is little doubt that patients may acquire extraordinary tolerance to the arsenicals, as exemplified by the remarkable case of Photinos,[120] in which a woman patient was given a total of 191 gm. of neoarsphenamine in 230 injections of which 190 were 0.9 gm. each, during a period of 6 years. As a general rule, the percentage of persons having reaction rises from the sixth to the tenth injection, and then remains more or less stationary up to the sixteenth to twentieth injections. From this point there is a further rise, which then declines to a low point between the thirty-first and the thirty-fifth injection. Harrison[64b] and Cole and his co-workers[30] have both ob-

served severe reactions often from the seventh to the fifteenth dose. The Coöperative Clinical Group[31a] found that reactions either of the mild or severe type occurred with the greatest frequency during the first course of treatment, and dropped off gradually with each succeeding course. Waugh and Milovich[179] found that 87% of the crustaceous dermatitis reactions occurred less than 2 months after the beginning of treatment. Twenty-two per cent followed 1 to 6 arsenical injections. In the Coöperative Clinical Group material 67% of the crustaceous dermatitis manifested itself before the second month of treatment; 53% following 1 to 6 injections, and 14% following 7 to 12 injections. In Waugh and Milovich's series, men received an average of 11 injections of arsphenamine and the women 13. Burke[18] believed from his experience that postarsphenamine blood dyscrasia is unrelated to dosage but that it tends to appear late in the course of treatment. Our experience corresponds to that of Burke. It is probable that in this and some other types of reaction (*e. g.*, hepatic) subthreshold injury begins long before the symptomatic reaction appears and can be detected by appropriate tests (*e. g.*, blood smears and counts, icterus index).

Type of Syphilis and Incidence of Arsenical Reaction. The Coöperative Clinical Group[31a] found that in early syphilis, the rate for mild reactions was 3 times higher than for severe reactions. Severe reactions, however, showed their highest incidence in primary syphilis followed by a lessened incidence in secondary and latent syphilis. Waugh and Milovich's[179] report showed the incidence of severe reactions to be about the same in all the earlier stages of syphilis; being 2.4 per 1000 arsenical injections for primary syphilis, 2.69 for secondary syphilis and 2.41 for latent syphilis. They also found that the incidence was considerably higher for the white patient with primary syphilis and slightly higher for white patients with secondary syphilis. In latent syphilis the incidence for the colored was slightly higher than that for the white patient. Cole and his associates,[30] in their attempt to determine whether the stage of syphilis during which the patient began treatment influenced the type of complication occurring, found that among the early cases the incidence of gastro-intestinal reactions was 27.7%, whereas among the latent cases the percentage was nearly doubled—50.6%. A much higher percentage of patients with early syphilis suffered slight skin reactions than of those who began treatment while in the latent stages. There were twice as many nitritoid reactions among the latent cases as among the early cases. Patients with treatment-resistant syphilis have been found to be specially reactive to the arsenicals by Jessner[76] and by Beerman.[13c] This fact may be indirectly concerned in the production of treatment-resistance since a reacting patient can only be given smaller doses which in turn may produce treatment-resistance.

Effect of the Type of Arsenical Drug on Reaction. Excluding such factors in individual drugs as certain metallic elements (*e. g.*, silver in silver arsphenamine which may produce argyria,[22a,121] there are a number of differences in the reaction tendencies of the various arsenical drugs. As in any of the factors discussed in this review, this one factor of drug type cannot be used in itself to explain the incidence of reactivity of a given group of patients to the arsenical. The experience of

the Coöperative Clinical Group[31a] revealed that for mild reactions the rate per 1000 injections was practically identical for old arsphenamine and neoarsphenamine, somewhat lower for silver arsphenamine, sulfarsphenamine and Bismarsen (bismuth arsphenamine sulfonate). The incidence of severe reaction following sulfarsphenamine was 3.5 per 1000—a higher rate than that for arsphenamine or neoarsphenamine, due to the disproportionately high incidence of crustaceous dermatitis and purpura hæmorrhagica. Severe reactions from old arsphenamine (606) were no higher than from neoarsphenamine. The complexity and confusion inseparable from the reaction problem is well illustrated by the contrasting experience with sulfarsphenamine (used from 1925 to 1939) of the United States Naval Medical Department.[152a] Among 29,438 injections reported, there were in the Navy's experience only 17 mild, 8 severe, and no fatal reactions to sulfarsphenamine. During the year 1939, 943 doses of this drug were administered with no reactions whatsoever. In the 15-year period the incidence of reactions to 1178 doses of sulfarsphenamine is somewhat higher than the average ratio of 1 to 1470 doses computed for 8 arsenicals, trivalent and pentavalent, used by the United States Navy. On the other hand, E. T. Burke, in his study of blood dyscrasias,[18] maintained that although all sulfarsphenamines are much more prone to give rise to dermatitis and blood dyscrasias than are arsphenamine, neoarsphenamine or arsphenamine diglucoside, certain brands of sulfarsphenamine are much more likely to cause this particular reaction. He believed that the variation in the toxic effects observed in the use of different brands appears to be related to molecular structure and this, in turn, to process of manufacture. He appealed for a greater degree of standardization in this reported group of remedial agents. Just as the different arsenicals or brands of arsenicals, or even of lots of the same arsenical, vary in therapeutic potency, so may they vary in reaction-producing tendency. Sulzberger and Simon[166] noted in connection with the differing qualities of various lots and brands of neoarsphenamine that there was a varying sensitizing proclivity in man as well as in animals, for the various drugs. Cole and his associates,[30] in discussing the comparative number of complications among patients treated with arsphenamine and those treated with neoarsphenamine, found little or no difference in the number of reactions as between the two drugs (15% reaction for arsphenamine and 16.7% for neoarsphenamine).

Mapharsen (arsenoxide), it will be recalled, has as one of its strongest appeals and recommendations an exceptionally low incidence of reaction. Epstein,[41d] compiling data from 92,000 Mapharsen injections from his own experience and the literature, found skin reactions, including exfoliative dermatitis, from one-half to one-third or even only one-ninth as frequent as with the other arsenicals. This was especially true of exfoliative dermatitis.

In common with Appel,[3b] Jordon and Traenkle,[77] Cole and Palmer,[29] Astrachan and Wise,[7] Epstein[41d] therefore recommends the use of Mapharsen in patients intolerant to other arsenicals. He suggests, in view of the experience of Parsons,[118] Foerster and his associates,[48,182] Kulchar and Barnett,[84] and Marshall,[99] all of whom reported recurrences of crustaceous dermatitis after the intravenous injection of Mapharsen or a pruritus and erythema after such an event, that patch

tests be used before the administration of Mapharsen to previously dermatitic patients. Fatalities[81,123,126,143,145] to Mapharsen are extremely rare. Mention has already been made of the lessened toxicity of the Mapharsen when used in the continuous intravenous drip method of massive arsenotherapy as compared with neoarsphenamine. On the basis of lessened toxicity, Rein and Wise[126] have found arsenoxide (Mapharsen) a convenient medication for treatment of syphilis in office practice. In addition Mapharsen has been advocated, contrary to our considered opinion unless under expert direction, as a substitution product for neoarsphenamine and other arsenical drugs in various of the severe reactions. Acetyl-glycarsenobenzene (Solu-salvarsan)[61,64a] has been mentioned previously along with Thio-Arsene[11,32,40,129] as drugs which have been abandoned in this country because of high incidence of reaction. Trisodarsen's[154a] reaction incidence is under investigation, though Givan and Villa,[52] employing this drug in congenital syphilis, reported it well tolerated by intravenous and intramuscular routes. Bismarsen (bismuth arsphenamine sulfonate)[60a,111,159,167,171] has a low incidence of reaction and in selected cases can be used as a substitute for other arsenicals to which the patient is reactive. The incidence of hematopoietic accidents is low, though a few recent cases have been reported. Sulfarsphenamine, silver arsphenamine, neoarsphenamine and arsphenamine have been adequately discussed in Part I of this review.

Diet, Nutritional State as Factors Influencing the Occurrence of Arsenical Reaction. In the past decade, and especially in the past 6 years, much progress both clinically and experimentally has been made on the effect of diet and other nutritional states on the background of arsenical reaction. There have, of course, been impressions that starvation, impaired nutritional states induced by the blockade in the First World War, poverty and other factors predisposed patients to treatment reactions from the arsenicals. It is now clear, however, from studies beginning with that of Craven,[35] substantiated by Schifrin,[138] and recently further confirmed by Messinger and Hawkins,[102] that for the liver at least, diet plays a major rôle in the prevention of damage by the arsphenamines. Craven[35] showed that dogs on a high fat and high nitrogen diet obtained maximum protection against liver injury resulting from the administration of arsphenamine. Of these two diets, Craven found that the former was more advantageous. A high carbohydrate diet, on the other hand, led to maximum susceptibility to liver injury caused by arsphenamine. He also indicated that starvation is an important factor predisposing toward liver injury caused by the arsphenamines. Cystine added to the diet or given intravenously did not increase the protective action of a carbohydrate diet. Schifrin[138] came to much the same conclusion as Craven, with reference to the failure of carbohydrate diets to protect the liver against injury from arsphenamine, even when a large amount of glycogen was demonstrable in the liver. He found, however, in contrast to Craven, that a high fat diet also left the liver sensitive to the ill-effects of arsphenamine; but that a diet rich in albumin was the most effective in preventing liver injury by this agent. Beerman,[13b] using rats instead of dogs, could not confirm or disprove the contentions of either Schifrin or Craven. In 1940, Messinger and Hawkins[102] found protein to be

most effective in protecting dogs against arsphenamine liver injury. On a protein diet the liver injury was trivial and promptly repaired. They, however, found a carbohydrate diet also beneficial but not as uniformly protective, and liver injury following this latter diet, when it occurred, seemed more severe. Fat proved to be deleterious and the fat-fed arsphenamine-treated dogs showed markedly progressive jaundice, severe liver injury and intoxication to the point of death.

Sulzberger, working with Mayer,[164] Simon[166] and others, on experiments with arsphenamine sensitization in guinea-pigs, found that guinea-pigs could be sensitized by intradermal injections of neoarsphenamine with great ease and regularity in Breslau, Germany; Boston, Mass.; Zurich, Switzerland; Odessa, Russia; and Ottawa, Ontario. But with the same technique, when fed on New York fodder, it was practically impossible to sensitize New York guinea-pigs in New York City. Sulzberger and Simon believed the cause of this variation in susceptibility to sensitization was not racial or constitutional, for New York guinea-pigs when transported to Breslau and Zurich could there be sensitized with ease. Sulzberger and Mayer believed that green fodder (alkaline ash) inhibited and dry fodder favored sensitization. Further observations reported by Sulzberger and Simon suggested that different lots of neoarsphenamine may have been factors in varying sensitizing effects as measured by a so-called sensitization index in men as well as in animals. They believe that this index is independent of the actual toxicity of the arsenical as such.

In the past 7 years beginning with the experimental work of Sulzberger and Oser,[165] there has been much interest in the question of relationship of vitamin C (cevitamic acid) to various types of sensitivity. The problem has been studied by a number of workers in connection with arsenic sensitivity in animals and in man. Certain of these investigators found that vitamin C, administered by various routes,[14,34d,h,37a,b,39b,47,86,104,127,162,165,174,176] helped to prevent reaction to arsenicals while certain others[25,27,42,50,94,178] have not been so convinced of the value of this vitamin as a reaction inhibitor. Durel[39b] furthermore believed that using vitamin C in solvents for arsenicals reduced the therapeutic effectiveness of the drugs. This was denied by Dainow[37] and partially by Cormia.[34h] This subject is in such a state of flux that it is impossible at the moment to say definitely whether vitamin C, administered orally or by parenteral route, has any significance in the reduction of arsphenamine reactivity. On the other hand, there is accumulating evidence to suggest that such a relation does exist, insofar as the use of vitamin C-containing solvents for neoarsphenamine administration has proven effective in reducing neoarsphenamine toxicity. Furthermore, Cormia[34h] presented convincing evidence of the influence of vitamin C in arsphenamine sensitiveness, by giving 6 patients who had recovered from definite arsphenamine dermatitis, massive doses of vitamin C (500 mg. intravenously daily for 7 days). After a cautious intravenous test with minute doses of the same brand of arsenical which had produced the cutaneous reaction, 5 of the 6 patients were eventually able to take full doses of the arsphenamine without further cutaneous reaction. The patch test previously positive, became negative in 3 of 4 patients subsequently tested. Cormia kept

his patients' vitamin C levels high by daily oral administration of 100 to 200 mg. of the vitamin.

Wien[183] also made a study with large numbers of mice, to see the effects of simple variations in an ordinary laboratory diet on resistance of mice to mercurochrome and neoarsphenamine. His results confirmed the belief that in mice, the toxicity of the drug is among other things dependent on diet. He was able to demonstrate, for example, that in toxicity determinations of neoarsphenamine preparations made on various diets, different values were obtained for the average lethal dose under different diets. He suggested that toxicity determinations for arsphenamine preparations be made simultaneously with the standard brands.

Nervous States and Reaction to Arsenical Therapy. The influence of nervous factors on any of the arsphenamine reactions is difficult to evaluate. Suggestion, apprehension, and undue emphasis on the likelihood of reaction on the part of the treatment-room technician or physician at times seem to increase the occurrence of the immediate reaction. The mechanism for the production of such effects at least for cutaneous reaction has been discussed elsewhere.[154,158] In addition, the repetition of visits to the clinic or office with the concomitant repetition of the same emotions which the patient experienced at the time of the first visit, when the diagnosis of syphilis was made, may lead to a heightened emotional state on the part of the patient. Eventually, prolonged therapy may lead to what Cormia[34g] has called the overtreatment syndrome, in which the patient complains of a striking degree of nervous irritability. Occasionally patients with this syndrome produced during arsphenamine treatment developed actual arsenical reactions, such as herpes zoster, nitritoid crises, and thrombocytopenic purpura.

Seasonal Influences on Arsenical Reactivity. Mention has already been made of Sulzberger's, Mayer's and their associates' work on the effect of differing geographic areas as factors in the sensitization proclivities of various arsenical drugs. This work, to some extent, is corroborated from the standpoint of the effect of sesons by the studies of Akiyama and his associates[1] who found among the reactions occurring in 3016 cases treated with arsphenamine in the period from 1921 to 1937, that the arsphenamine exanthemata were seen most frequently in January and June.

The Coöperative Clinical Group[31a] made observations of the effect of different years in which the treatment was given on the incidence of treatment reactions to the arsenicals. The rate per 1000 injections for severe reactions dropped in all the clinics progressively from 1920 to 1931 inclusive. On the other hand, with mild reaction, after a great increase in the year 1921, the rate ran about level until the period 1926–29 when there was a pronounced increase which later dropped to the lowest level of the entire period. This increase in this 5-year period seemed to be explained as due to greater care in observing and recording mild reactions. Perhaps because of increased attention to mild reactions, the rate of severe reaction fell to a level of only 1.6 per 1000 injections in the years 1929–31 as against 3.1 in the years 1920–22.

From 1925 to 1933, in a comparison of the yearly incidence of postarsphenamine and infectious jaundice, Wile and Sams[184] noted that

nearly 60% of their cases of late postarsphenamine jaundice occurred during the years 1930, 1931 and 1932. At the same time about half of the cases of infectious jaundice treated in the hospital from 1925 to 1933 occurred. Ruge[131] and Stokes, Ruedemann and Lemon[160] had previously noted a close parallelism between the occurrence of postarsphenamine jaundice and infectious jaundice. In connection with seasons, the incidence of intercurrent infection at various times of the year probably plays a part in the frequency of reactions and in their severity when they occur.

Wile and Sams[184] in their study of jaundice in syphilis found that there was a tendency for late jaundice to be slightly more common in the winter months, but this is not so marked as with infectious jaundice. This tendency has been previously noted by others (Todd,[172] Stokes, Ruedemann and Lemon,[160] Ruge[131]). Stokes, Ruedemann and Lemon definitely showed that their cases of jaundice were greatest in number during the months of respiratory and systemic infections, and approximately half the number had associated upper respiratory tract disturbances in the prodromal stage.

Pregnancy and Toxemia of Pregnancy in Treatment Reaction. The normal effect of pregnancy on the various organs of elimination would lead one to suspect that any other extraneous influence with potential hepatotoxic and nephrotoxic action would increase greatly the likelihood of reaction to the arsenicals in pregnant women. Furthermore, the question of whether arsenicals given to pregnant women could possibly increase the likelihood of the so-called toxemia of pregnancy needs fuller investigation. Several recent discussions of the subject, particularly that of the Coöperative Clinical Group,[31b] by McCord,[93] and by Cole,[28] convey the impression that pregnant syphilitic women tolerate arsenical therapy well. McCord has apparently never seen a serious treatment reaction or death in the medical supervision of more than 2000 syphilitic pregnant women. On the other hand, Ingraham,[72] in a carefully documented study of the complications due to arsenical therapy in syphilitic pregnant women, recorded 7 maternal deaths, the study being based largely on data obtained from antisyphilitic therapy reported from six different sources to the Committee on Maternal Welfare of the Philadelphia County Medical Society, since 1931. He was able in addition to collect 35 more deaths from the literature which he believes indicate that the pregnant syphilitic woman is not exempt from any of the severer types of treatment reaction, such as hemorrhagic encephalitis, acute circulatory collapse, damage to liver, arsphenamine dermatitis and aplastic anemia, all of which have been causes of death. He believes, moreover, that there is evidence to indicate that antisyphilitic treatment may aggravate an already existing toxemia of pregnancy, or precipitate an incipient one. Moore[105] has taken exception to Ingraham's findings and in a study sponsored by Moore and made by Peckham,[119] it was shown that in a series of 13,742 consecutive deliveries at the Johns Hopkins Hospital, the incidence of toxemia of pregnancy was somewhat lower in syphilitic than in non-syphilitic patients. No correlation could be established in the study between the stage of syphilis at the time of its diagnosis and treatment during pregnancy and the frequency of toxemia. Peckham concludes that antisyphilitic treatment admin-

istered to syphilitic women during pregnancy does not increase the incidence of toxemia of pregnancy. The question of the influence of pregnancy on treatment reaction is still open, but the Reviewers feel that conservative individualized therapy during pregnancy with careful attention to detail will yield little or no excess of untoward reaction, in spite of the fact that individual isolated cases of serious reaction may occur in spite of careful control.

Rôle of Intercurrent Infection in Untoward Reaction to the Arsenicals. While this subject has been best worked out in connection with cutaneous reaction, undoubtedly the principles can be extended to apply to other types of reaction occurring in patients receiving arsenicals who are subject to intercurrent disease, fungus infection, or foci of infection. The rôle of intercurrent infection in untoward reactions to antisyphilitic treatment can be divided according to Dudley Smith[144] into two parts: 1, the harmful effect of antisyphilitic drugs on other infections; and, 2, the part which intercurrent infections play in producing harmful reactions in syphilitic persons. In 1921 Moore and Keidel[106] published an extensive study relative to the appearance of various skin diseases, including exfoliative dermatitis and other less complex reactions such as urticaria, erythema, herpes simplex and so forth following antisyphilitic treatment with arsenicals. They intimated that the secondary dermatoses like herpes could possibly result from something more than the actual local toxic effects of the arsenic, and were inclined to accept Milian's contention[103] that certain eruptions produced by the arsenicals are like acute exanthemata such as measles, and due to a lighting up of latent bacterial infection. Stokes and Cathcart[156] were pioneers in calling attention to the influence of focal and intercurrent infections on arsenical dermatitis. In 1934 Stokes and Kulchar[157] extended the concept to include dermatophytosis. According to these authors a part of the influence of infection was probably exerted through the allergic mechanism of arsphenamine sensitization, and involved the conception of multiple balanced and summation effects as suggested by Vaughan[175] in general, by Harkavy and Hebald[63] for infectious asthma and arthritis, and by the observations of Moore, Woo and Robinson[107] on the reactivity of atopics to arsphenamine intradermally. When the infection involves the skin in antecedent or induced dermatitis from other causes, the local dermatitic focus acts as an excitant to the general flare-up following arsenical medication. Dermatophytic infection may apparently act as a participant in a balanced or a "summation" allergic complex involving an arsenical, an arsenical extending the range and increasing the severity of the allergic dermatitic response to the drug. A dermatophytic focus may also conceivably serve through the induction of local vasodilatation as the starting point of an "arsenical" dermatitis. Minute amounts of arsphenamine in arsphenamine-sensitive patients may flare up and generalize a local, apparently mycotic, dermatitis. The interaction of a drug on infection allergy may be reversible, and may in some cases take the course of Milian's "activation" of the infectious focus with the appearance of an exanthem of the toxic erythema type and a flare-up of the local focus in the case of bacterial infection, or an exacerbation of a localized mycotic process or a "mycotid" in the case of a fungus infection. Their experience led Stokes and Kulchar[157] to suggest that in dealing with an arsphena-

mine dermatitis in patients with focal infective lesions, mycotic or bacterial, treatment should be directed, albeit with caution, at the focal as well as at the general process. In 1937 Stokes and Callaway[155] expressed the opinion that intercurrent infections like influenza may exercise a sensitizing influence to pyogenic and mycotic dermatoses as well as to light. Smith, in his study, quotes a number of observers, one of whom, Weiss[181] pointed out in 1925 that fatal accidents following antisyphilitic treatment sometimes can be prevented if caution is observed in treating patients with acute infections, especially pneumonia. The pneumonia induction may be in part due to mechanical and allergic effects involving the pulmonary circulation. He also cited Cormia[34a] (1936) in his work on experimental arsphenamine dermatitis which showed that staphylococcus toxin did not increase the incidence or severity of the reactions to arsphenamine in guinea-pigs, and similar results were observed in guinea-pigs infected with streptococci. Smith comments that this does not reproduce the situation found in intercurrent infections in man. Netter and Urbain[110] felt that from their studies with the virus of herpes zoster that arsphenamine as well as other agents is responsible for cases of secondary zoster because the drug possibly stirs up to activity a latent virus in the ganglia. Much of the jaundice which occurs during arsenical therapy and is attributed to the tonic effects of the drug is in all probability the expression of an epidemic of infectious hepatitis. (Stokes, Ruedemann and Lemon;[160] Ruge;[131] Wile and Sams;[184] Lane[87] and others.)

Technical Elements in Reaction Production. It is not intended under this heading to discuss such well-known causes of reaction as the use of deteriorated drugs, aëration of neoarsphenamine through shaking, improper alkalinization of arsphenamine and so forth.

In 1931 Hirshfeld, Hyman and Wanger[65] produced and described "speed shock." They demonstrated that the rapid intravenous introduction of pharmacologically active or inert chemicals, drugs and biologic fluids may frequently give rise to immediate and far-reaching non-specific sequelæ, at times serious and occasionally fatal. The clinical syndrome of "speed shock" occurs 40 to 60 seconds after injection. The reaction was apparently not a species idiosyncrasy since it could be obtained in cats, dogs, monkeys and man. Anesthetics and narcotics were unimportant factors in production since the syndrome was produced in unanesthetized animals. The antidote to speed shock was a slow infusion or intravenous "drip," which was developed for various purposes and lately introduced into chemotherapy. Hyman and his colleagues[68,69a,b,70] felt that if the concept of "speed shock" were correct and the immediate toxic effect that followed the introduction of chemotherapeutic agents were technical rather than pharmacologic, potent therapeutic agents might be administered in "doses far greater than at present employed, and this without serious damage to the cells of the host." This concept has reached fruition in the intravenous arsphenotherapy. Hyman, in an interesting study on the subject,[68] compared the toxic phenomenon of the intravenous massive dose chemotherapy with routine treatment. Contrary to expectations he states that there do not appear to be late toxic complications arising from excessive dosage or accumulation, although difficulties due to idiosyncrasies do crop up unexpectedly causing treatment morbidity

and treatment mortality. The incidence of these closely approximates those of routine therapy and because the results of therapy approach the goal of the chemotherapist, Hyman believes they would seem to justify the hazards of increased toxicity if present.

The incidence of reaction in massive arsenotherapy has been well studied by the Baehr-Chargin group.[10] In his recent compilation of the toxic manifestations, Chargin illustrates the complexity of this problem of dosage and reaction incidence. He states that in previous experience with 111 patients treated by the continuous intravenous drip method with neoarsphenamine, there was 1 fatality due to hemorrhagic encephalitis and 1 recovered case, and a rather high incidence of polyneuritis (38%). This led to the substitution of Mapharsen for neoarsphenamine, which subsequently proved to be less toxic and possibly equally efficacious therapeutically. This substitution of one drug for another is not as simple an explanation as it would seem on the surface, for the cause of the difference in toxicologic manifestations, inasmuch as the Mapharsen was used in a dosage much lower than that previously employed in the case of neoarsphenamine (1200 mg. Mapharsen as compared with an average dose of 4 gm. (4000 mg.) neoarsphenamine). From the experimental side, however, Magnuson and Raulston[98] found that the maximum tolerated dose and the minimal lethal dose are such as to indicate the need for considerable caution in the administration of massive doses of Mapharsen to patients by the continuous drip method.

In a preliminary report on the rapid intensive treatment of early syphilis with multiple injections of Mapharsen, comprising 275 cases treated with Mapharsen alone and 141 cases treated with Mapharsen and fever, Thomas and Wexler[169] showed that the combination of intravenous typhoid vaccine and Mapharsen, especially when both are administered the same day, give more frequent transitory toxic effects than Mapharsen alone, but no clinical evidence of hemorrhagic encephalitis developed in 141 cases. By reducing the number of fever bouts and omitting Mapharsen the days when fever is given they believe the occurrence of even minor toxic effects can be materially reduced. Cannon[22b] using a simplified syringe technique for administering old arsphenamine (606) gave 16,943 injections to 1187 patients (1053 adults and 134 children); 77% of these injections were well tolerated.

Another technical consideration which has recently been pointed out in connection with new drugs but for which there is no apparent explanation, is the rate of injection by the syringe method of neoarsphenamine as compared with Mapharsen. The former must be injected slowly in order to avoid reactions, whereas Mapharsen is better tolerated when injected by rapid procedure.

Consideration of Individual Types of Reactions to the Arsenicals: Cutaneous Reactions. New types of cutaneous manifestations have been recently reported which deserve only mention, as follows: vesicobullous dermatitis,[108] unusual reticular fibro-atrophoderma,[41c] and vitiligo.[23] Fixed eruptions due to the arsphenamines have been thoroughly reviewed by Chargin and Leifer[26] whose series (69 patients) seems to be the largest recorded. Of these 69 receiving one of the trivalent arsenicals, the drug was clearly responsible for the fixed eruption in 68. Chargin and Leifer felt that race was an important pre-

disposing factor inasmuch as 79% occurred in negroes, 19% in whites, and 2% in the yellow races. The lesions were most common on the face and were also observed over the entire body, but not on the scalp, palms and soles. The mucous membrane of the mouth was involved in 2 cases. In descending order of frequency the precipitating drugs were arsphenamine, neoarsphenamine, silver arsphenamine, Mapharsen, and Trisodarsen. Lesions produced by one trivalent drug may be activated by other trivalent drugs and also by some pentavalent drugs. Activation only follows the use of the intravenous or intramuscular route. Patch tests and iontophoresis have been consistently negative. Full-thickness autotransplants of the involved skin in 3 patients failed to react when the drug was readministered. None of these cases progressed to exfoliative dermatitis or any other type of arsenical dermatitis in spite of continued arsenical therapy. Since all trivalent arsenicals do not produce fixed eruptions with equal frequency, it is possible to continue therapy uneventfully by changing the type of arsenical.

While according to the work of Osborne and his co-workers[114a,b,115] (*cf.*[168]) the trivalent arsenicals and the pentavalent arsenicals have a different point of attack in the skin, reports have now appeared which would tend to indicate that the possibility of sensitization to both trivalent and pentavalent arsenicals in the same patient is possible. This concept was exemplified by the cases of Epstein,[41b] Golz[58] and Franks and Fisher.[49] This observation should lead to caution in further arsenical therapy with a pentavalent compound in a patient who has once recovered from a reaction to a trivalent arsenical compound.

Another cutaneous reaction to arsenical therapy worthy of mention is the so-called erythema of the ninth day. This cutaneous reaction has been redescribed by a number of individuals, including Keim,[80] Epstein and Lævin,[43] Goldman and his associates,[56,57] Sprafke,[148] Peters (Am. J. Syph. Gonor. and Ven. Dis., **25,** 527, 194), and others.[21,60b] This eruption, originally designated by Milian as "erythema of the ninth day," is an entity which should be differentiated from the exfoliative dermatitis of arsenical causation and certain acute exanthems. The mechanism of this reaction is unknown. Milian considered it a toxic erythema from the flaring of a latent non-syphilitic infection focus by the drug. Other speculations include a supposed response of the autonomic nervous system to either the dose or the type of trivalent arsenical or the mode of injection. The eruption is usually acute, coming on 7 to 10 days after the institution of arsenical treatment, and thus usually with the second injection. The onset suggests that of an acute infection with chills and fever 24 hours after the injection. From about the second to the fourth day the eruption appears and may be indistinguishable from that of measles or even a rather coarse type of scarlatiniform erythema. Edema of the eyelids often occurs but there is usually no coryza. The comparatively small degree of involvement of the mucous membrane without Koplik's spots assists in differentiation. This process is self-limited and may or may not be associated with a variety of complaints. Further arsenical treatment is not contraindicated.

The newer conceptions of the clinical aspects of postarsphenamine dermatitis have been sufficiently described (Stokes,[153] Sulzberger,[163] Cormia,[34b,e,f,i,j] E. Epstein[41a] and many others). Whether or not arsenical therapy can be continued after this complication is always a

critical question. Patch tests with the arsenical have proved unreliable (Beerman[13d]) and the possibilities of the harmful effects from them have been pointed out by Bechet,[12] Beerman,[13a] and Schoch.[139a,b] Cautious testing of tolerance by intravenous testing of minute doses has been proposed by Robinson.[128] Schoch and his collaborators[140] sidestep the difficulty by substituting Mapharsen successfully for the arsphenamine in 40 cases. The initial dose for the tolerance test should be 1 mg. Even minute doses are not tolerated by patients who have once sustained a severe exfoliative accident after neoarsphenamine. Schoch and his co-workers found that patch-testing with Mapharsen (3.3% solution) is a more reliable test of tolerance than the corresponding concentration of neoarsphenamine in patch-tests.

Liver Damage Due to Arsenical Compounds. The pharmacologically known hepatotoxicity of arsenic has led to a good deal of post-hoc thinking on the hepatic reactions to arsphenamine and other antisyphilitic arsenical treatment. When a patient under treatment with an arsenical develops jaundice, not less than six, and probably several more possibilities must be considered. It has become increasingly clear that epidemic waves of postarsenical jaundice, many cases occurring months and sometimes years after the exhibition of the drug, are in reality catarrhal infective accidents to which the drug may or may not have served as a predisposing cause (Stokes, Ruedemann and Lemon,[160] Wile and Sams,[184] Sager.[134] (See also [5,20,51,59,62,74,75,85,87,96,112,124,131,146,147,180].) The entire subject has recently been thoroughly reviewed by Lane[87] on the basis of a series of 100 of his own cases, from which he concludes that syphilis alone seems a relatively rare causative factor; that arsenic alone does not explain the complication, though it exerts an influence in most cases; that bismuth appears to participate in some degree; that tryparsamide can produce jaundice as well as the trivalent arsenicals, and that death may occur late from hepatic cirrhosis. The discussion of this paper supported in general the conception that antisyphilitic treatment is a contributing but not a sole factor in the induction of hepatic injury. Soffer[146,147] detected racial differences (whites three times as frequently jaundice as negroes), but no influence from the stage of the syphilitic infection or the stage of treatment. Gott and Doyle[59] confirmed the racial difference, and found that Mapharsen produces jaundice 10% less frequently than neoarsphenamine. The possibility of minute amounts of arsenic in preparations credited with the production of jaundice has been investigated by Russell,[133] and Lowenburg and Naide[91] with negative results.

Hemorrhagic Encephalitis. Variously named pericapillary encephalorrhagia (Globus and Ginsburg[54]), brain purpura (Alpers[2]), and arsphenamine encephalopathy (Levy[88]), this occurs "normally" once in every 5398 treated cases, or 1 case in every 28,758 injections of an arsenical (Glaser, Imerman and Imerman[53]). The occurrence of the reaction like a bolt from the blue, virtually without warning, and accompanied by an exceedingly high mortality, make it one of the most disturbing complications of treatment for syphilis. Glaser and the Imermans point out that the reaction occurs in non-syphilitic persons, apparently without relation to age or sex, or to the dosage of the drug administered, to the number of injections given, or to the intrinsic toxicity of the preparation. The reaction occurs most commonly after

the second dose; may develop within 12 hours or after a latent period as long as several days. It was formerly almost invariably fatal. The prognosis has, however, steadily improved in recent years through the use of repeated lumbar puncture, sedation, adrenalin, and especially dehydration, including intravenous hypertonic solution. A prodrome of confusion with conduct abnormalities may permit the recognition of an incipient case before a second injection is given, and a fatality be thus avoided. The autopsy picture in the brain is that of dilatation and engorgement of meningeal and cerebral vessels with a picture suggesting "wet brain," plus vascular necrosis and perivascular hemorrhage of the "ringed type." (See [16,34c,83,116,117,130,132,173a,b].)

Hemolytopoietic Reaction to Arsenicals. Enough interest has been aroused by hematopoietic damage attributed to arsenicals in the treatment of syphilis to provide the basis for a separate review. (See [9,15,17,36,44,45,46,66,67,71,78,79,81,89,90,92,101,135,137,161,170,185,186]). Burke's views have been alluded to. Every drug now in use in the treatment of syphiliis, including Mapharsen, has been shown to be capable of producing hemolytopoietic reaction, so that the substitution of one drug for another, as suggested by Falconer and Epstein and their group[42,44,45] as well as Goldberg[55] is certainly not free from risk. Epstein and Falconer[42] in studying 8 patients who had had a thrombocytopenic purpura following neoarsphenamine or Bismarsen, were able to show that very minute amounts of the offending drug or a related member of the group, was capable of reviving the thrombocytopenic reaction, and that successive reactions led to increasing sensitiveness to the drug, so that repetition of treatment after evidence of thrombocytopenia had once developed, is definitely and indeed exceedingly dangerous. Vitamin C was employed in large doses and in the diet in an attempt to control the thrombocytopenic reaction, without effect. Subthreshold hemolytopoietic reaction was clearly recognized in 30 patients who, although they tolerated antisyphilitic treatment without clinical reaction, sustained diminution of leukocytes and platelets in the peripheral blood following each treatment. Mapharsen appeared less likely to produce these changes than neoarsphenamine. The simultaneous use of two drugs known to influence the hemolytopoietic system is a matter for concern in patients simultaneously treated for syphilis and gonorrhea with an arsenical and a sulfonamide. Caletti[19] observed no ill-effects from such synchronous use of drugs with known hematopoietic effect, and concludes that intolerance is a matter of the individual drug, and not of synergistic use.

New Methods of Reaction Prevention. Doak,[38] Appel[3a] and Jankelson[4] and also Shaw[142] have contributed important studies. Doak in particular reviewed the use of sugar, calcium salts, degradation products of protein including the aminoacids and other substances and compounds recommended for admixture with the arsphenamine solution, before administration.[39a,82,97,125,141,177] The question of the efficacy of these substances remains open. The possible influence of vitamin C previously mentioned is supported and opposed by about equal numbers of observers. MacKee and Astrachan[6,95] who over a period of years have employed liver extract as a detoxifying agent in intolerance to arsenicals, heavy metal and irradiation, have felt increasingly certain that liver extract is a valuable detoxifying aid. Sodium thiosulfate has been

the subject of a number of studies and publications by Ceder, Zon, and Klinger,[24] Ayres and Anderson,[8] Muir, Stenhouse and Becker,[109] Oppenheim and Fantl,[113] and Mattice and Weisman.[100] Ceder and his associates[24] conclude that while there is still room for controversy, much of the disparity of opinion is due to artefacts and technical difficulty incident on arsenical analysis and on the selection of subjects. In a study of 10 patients under treatment with an arsphenamine and in whom urinary, fecal and total arsenic excretion was carefully studied, the 6 receiving sodium thiosulfate exhibited no significant differences from the 4 who did not receive sodium thiosulfate. It is concluded therefore that this drug, whose reputation has supposedly rested on its ability to facilitate the elimination of arsenic, if effectual at all, is certainly not so because of any influence on arsenic elimination. Clinical syphilologists have in general discarded sodium thiosulfate in the treatment of arsenical reactions.

HERMAN BEERMAN, M.D.
JOHN H. STOKES, M.D.

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Correction. In Dr. Sodeman's review of "Chronic Constrictive Pericarditis" in the July number of this journal (page 133), in the sentence reading "Dyspnea may occur on exertion, but in the absence of pleural fluid, orthopnea is present," the word "present" should be changed to "absent," so that the sentence will read that "orthopnea is absent."

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